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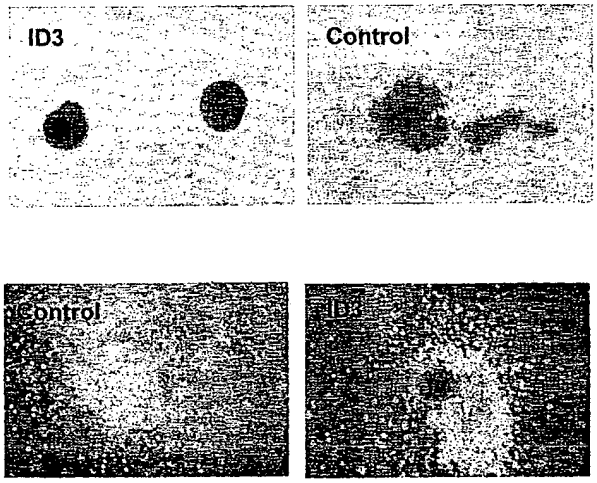
(54) Title: METHODS AND COMPOSITIONS FOR MODULATING STEM CELLS



(57) Abstract: The invention provides methods for inhibiting stem cell differentiation and for increasing the effective dose of stem cells in a subject. HSC differentiation can be inhibited by applying an HSC differentiation-inhibiting polypeptide identified in the present invention to an HSC culture in vitro, or administering the polypeptide to a subject in vivo. Some other methods of the invention comprise first obtaining a population of hematopoietic stem cells, introducing into the cells an HSC differentiation-inhibiting polynucleotide disclosed herein, and expressing the HSC differentiation-inhibiting polynucleotide in the cells. Such genetically modified stem cells can be administered to a subject whereby effective dose of the stem cells in the subject can be increased. This invention further provides novel molecular markers of hematopoietic stem cells, and methods for enriching hematopoietic stem cells using these novel markers.

METHODS AND COMPOSITIONS FOR MODULATING STEM CELLS

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of priority to U.S. Provisional Patent Application Serial No. 60/447,030 (filed February 12, 2003), the disclosure of which is incorporated herein by reference in its entirety and for all purposes.

FIELD OF THE INVENTION

The present invention generally relates to methods for enriching stem cell population and for modulating stem cell differentiation, as well as to therapeutic applications of such methods. More particularly, the invention pertains to genes differentially expressed in hematopoietic stem cells and to methods of using these genes to modulate stem cell differentiation.

BACKGROUND OF THE INVENTION

Hematopoiesis (hemopoiesis) is a process whereby multi-potent stem cells give rise to lineage-restricted progeny. The molecular basis of hematopoiesis remains poorly understood. Hematopoietic stem cells (HSCs) are the only cells in the hematopoietic system that produce other stem cells and give rise to the entire range of blood and immune system cells. These cells are able to self-proliferate, so as to maintain a continuous source of regenerative cells. When subject to particular environments and/or factors, they can differentiate to dedicated progenitor cells, where the dedicated progenitor cells may serve as the ancestor cell to a limited number of blood cell types.

HSCs and their progenies at the various development stages all play an important role in the normal function of the mammalian immune system. HSCs are of prominent therapeutic importance in many circumstances. In many diseased states, the disease is a result of some defect in the maturation process. In other situations, such as transplantation, there is a need to prevent the immune system from rejecting the transplant by irradiating the host. In neoplasia, a patient may be irradiated and/or treated with

chemotherapeutic agents to destroy the neoplastic tissue, which often also damage or destroy the host immune system. Further, other situations such as a severe insult to the immune system also result in a substantial reduction in stem cells and injury to the immune system. In all these situations, it will frequently be desirable to restore stem cells to the host. For example, HSCs are the active component in bone marrow transplantation (BMT), and transplant of highly purified HSC will completely restore the hematopoietic system in a manner indistinguishable from unfractioned bone marrow.

Despite decades of research, there are currently no satisfactory methods to expand the numbers of HSCs or accurately enumerate the numbers of expanded and engraftable HSCs cells following in vitro culture. There is a need in the art for better methods for isolating, enriching, and enumerating transplantable HSCs. The instant invention fulfills this and other needs.

SUMMARY OF THE INVENTION

In one aspect, the invention provides methods for inhibiting differentiation of mammalian stem cells. The methods entail (a) providing a population of stem cells, (b) introducing a vector comprising an HSC differentiation-inhibiting polynucleotide of the present invention into the stem cells, and (c) expressing a polypeptide encoded by the polynucleotide by culturing the modified stem cells, thereby inhibiting differentiation of the stem cells. In some of the methods, the stem cells are isolated from bone marrow. In some preferred methods, the stem cells are human hematopoietic stem cells. The human stem cells can be first selected for expression of CD38 and Thy prior to introduction of the vector. In some of the methods, the HSC differentiation-inhibiting polynucleotide encodes GATA-binding protein 3 or ID3.

In a related aspect, the invention provides methods for increasing the effective dose of hematopoietic stem cells in a mammalian subject. The methods require (a) providing a population of hematopoietic stem cells, (b) introducing into the cells an HSC differentiation-inhibiting polynucleotide of the present invention, and c) administering the genetically modified cells that express an HSC differentiation-inhibiting polypeptide to a mammalian subject; thereby increasing the effective dose of hematopoietic stem cells in the subject. In some of these methods, the administered stem cells are a subpopulation of the

modified cells that are selected for expression of the polypeptide prior to administering to the subject. In some preferred methods, the subject is human, and the hematopoietic stem cells are human hematopoietic stem cells. In these methods, the hematopoietic stem cells can be selected for expression of CD34 and Thy prior to introducing into the cells the HSC differentiation-inhibiting polynucleotide.

In another related aspect, the present invention provides methods for inhibiting hematopoietic stem cell differentiation using an HSC differentiation-inhibiting polypeptide identified by the present inventor. The methods entail contacting a population of HSCs with an effective amount of the HSC differentiation-inhibiting polypeptide which inhibits differentiation of the HSCs. In some of the methods, the HSCs are present in an in vitro cell culture. In some other methods, the HSCs are present in a subject grafted with the HSCs. In some preferred methods, the subject is human.

In another aspect, the invention provides methods for isolating a population of cells that are enriched for hematopoietic stem cells (HSCs). These methods comprise (a) obtaining a sample of cells containing hematopoietic stem cells, (b) selecting cells from the sample based on expression or lack of expression of at least one known HSC surface marker, and at least one novel HSC molecule marker identified in the present invention, and (c) separating cells with the known HSC marker and at least one of the novel molecule markers; thereby isolating a population of human cells enriched for hematopoietic stem cells.

Preferably, the hematopoietic stem cells enriched with these methods are human HSCs. In some methods, the known human HSC marker is CD34+ and Thy+. In some of the methods, the at least one novel HSC marker is a human HSC surface molecule identified in the present invention.

In another aspect, the invention provides methods for enumerating hematopoietic stem cells in a population of cells. The methods entail (a) contacting the population of cells with an antibody that specifically binds to one novel HSC surface marker identified in the present invention under conditions that allow the antibody to specifically bind to the HSC surface marker, and (b) quantifying the cells recognized by the antibody; thereby enumerating hematopoietic stem cells in the population of cells. In some of these methods, the hematopoietic stem cells are human HSCs, and the population of cells are first selected for expression of CD34 and Thy prior to the contacting.

A further understanding of the nature and advantages of the present invention may be realized by reference to the remaining portions of the specification and claims.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows schematic structure of expression vectors for overexpressing various HSC differentiation-inhibiting genes.

Figure 2 shows that ID3 over-expression increases the number of colony forming cells in CFC assay.

Figure 3 shows upregulated expression of various transcription factors in mouse HSCs.

DETAILED DESCRIPTION

I. Overview

The present invention is predicated in part on the discovery by the present inventor that a number of genes are differentially expressed in hematopoietic stem cell populations (see Examples below). It was also found that some of these HSC genes slow down HSC differentiation or enhance HSC activities when they are overexpressed in HSCs. These genes are therefore termed HSC differentiation-inhibiting genes.

Using HSCs enriched from blood of normal human donors, it was found that sequences upregulated in the human HSCs include genes encoding hormones, enzymes, histone, transcription factors, secreted proteins, surface markers, and other molecules. Table 1 lists examples of these genes that are upregulated in human HSCs (CD4+Thy+) as compared to non stem cells (CD4+Thy-). Further, using HSCs isolated from two different sources, bone marrow and peripheral blood, the present inventor identified a set of genes that are differentially expressed in HSCs from both sources. Some of these genes are shown in Table 2.

Similarly, in a mouse HSC population (CD34-CD38+), a number of genes encoding proteins with diverse biochemical and cellular functions were also upregulated, including genes encoding surface antigens, transcription factors or growth factors (see Tables 3 and 4). These novel HSC genes are enriched in HSCs compared to their

differentiated progeny (e.g., CD34+ CD38+ progenitor cells) or CD34+CD38- facilitator cells.

Without being bound in theory, the molecules upregulated in HSCs could play various functions in modulating HSC growth and differentiation, as well as regulating activities and functions of progenitor cells that differentiated from the HSCs. For example, increased levels of some of the surface receptors, growth factors, and secreted proteins shown in Table 2 could act in synergy in inhibiting HSC differentiation and promoting their expansion.

In accordance with these discoveries, the present invention provides methods for modulating HSC differentiation. Inhibition of HSC differentiation allows continued growth and expansion of the HSC population, and therefore provide engraftable HSCs with increased dosage and higher potency. A number of the upregulated HSC genes identified herein (e.g., shown in Tables 1, 3, and 4) can potentially function as HSC differentiation-inhibitors. For example, polypeptides encoded by the novel HSC genes disclosed herein (e.g., the growth factors or hormones shown in Table 2) can be used to inhibit HSC differentiation in vitro (e.g., by applying to an HSC cell culture) and in vivo (e.g., by administering to a subject engrafted with bone marrow or HSCs). Differentiation inhibiting activities of these molecules were exemplified by GATA3 and ID3 as shown in the Examples below.

As indicated by the GenBank accession numbers or other identification numbers or descriptions in Tables 1, 3, and 4, sequences of the upregulated human and mouse HSC genes disclosed herein are all known in the art. Thus, as detailed below, the HSC differentiation-inhibiting polynucleotide sequences can be easily obtained commercially, from the sources disclosed in the public databases, or isolated using routine techniques of molecular biology. The encoded polypeptides can also be obtained commercially or easily produced with standard procedures of recombinant techniques.

The invention also provides methods for isolating and enriching HSCs. The currently known HSC markers are not satisfactory because they cannot accurately predict homogeneity and hematopoiesis activities of cells bearing the markers. The discovery of genes differentially expressed in HSCs provides novel molecular markers for selecting and enriching HSCs. For example, antibodies against novel surface markers disclosed in the

present invention (e.g., those in Tables 2, 3, 4 and 5) can be used to isolate human and mouse HSCs from a crude population of cells (e.g., bone marrow or peripheral blood). The methods can also be directed to cell populations already enriched for one or more of the known HSCs makers (e.g., CD34+, Thy+ in human, and CD38+, c-kit+, Sca1+ in mice). Further enrichment using these novel markers can lead to more homogeneous HSCs with more potent hematopoiesis activities.

In both the autologous and allogeneic setting, the time to recover from BMT is directly related to the dose of HSCs transplanted. Even a modest 2 to 3-fold expansion of engraftable HSC would afford great benefit to patients by minimizing the duration of cytopenia when patients are most susceptible to infection. Thus, isolation and expansion of more homogeneous HSCs in vitro in accordance with the present invention would make autologous and allogeneic HSC transplantation safer and more effective.

The practice of the present invention will employ, unless otherwise indicated conventional techniques of cell biology, molecular biology, cell culture, immunology and the like which are in the skill of one in the art. These techniques are fully disclosed in the art, e.g., in Sambrook et al., "Molecular Cloning A Laboratory Manual," Cold Springs Harbor Laboratory Press (3rd ed. 2001); Carter and Sweet, "Methods of Enzymology," Academic Press (1997); and Harlow and Lane, "Antibodies, A Laboratory Manual," Cold Spring Harbor Press (1998).

The following sections provide more specific guidance for making and using the compositions of the invention, and for carrying out the methods of the invention.

Table 1. Genes upregulated in human CD34+Thy+ HSCs from peripheral blood

Classification	Name	Description
Histone	H2BFL	Homo sapiens H2B histone family, memberA
Histone	H2AFA	Human histone genes
Histone	H2A/l	Homo sapiens H2A histone family, member L
Histone	H1F2	Histone 2A-like protein gene
Histone	H2B/h	Homo sapiens H2B histone family, member H
Histone	HH2A/c	Human histone H2AFC gene
Histone	H2AFQ	Homo sapiens H2A histone family, member Q
HLA	HLA-DPB1	Human MHC class II lymphocyte antigen beta chain
HLA	HLA-DQB1	Human MHC class II HLA-DR2-Dw12 mRNA DQw1-beta
HLA	HLA-E	Homo sapiens HLA-E gene
Secreted-complement	PTS	Homo sapiens 6-pyruvoyltetrahydroprotein synthase
Secreted-complement	HFL1	Human factor H homologue mRNA complete cds
Secreted-growth factor	MDK	Homo sapiens midkine (neurite growth-promoting factor 2)
Secreted-hormone	OXT	Homo sapiens oxytocin, prepro-(neurophysin I) mRNA
Secreted-hormone	AVP	Homo sapiens arginine vasopressin mRNA
Signaling-GTP	R-Ras	Human R-ras
Signaling-GTP	GCHFR	Homo sapiens GTP cyclohydrolase I feedback regulatory protein
Signaling-GTP	GUCY1A3	Homo sapiens guanylate cyclase 1, soluble, alpha 3
Signaling-Kinase	WAF1	Human DNA sequence from PAC 431A14WAF1
Signaling-Kinase	ITPKB	Homo sapiens inositol 1,4,5-triphosphate 3-kinase B
Signaling-Kinase	PPKCL	Homo sapiens protein kinase C, eta
Signaling-Kinase	PPKCZ	Homo sapiens protein kinase C, zeta
Signaling-SH3	SKAP55	Homo sapiens src kinase-associated phosphoprotein of 55kDa
Stress	PTGS2	Homo sapiens prostaglandin-endoperoxide synthase 2
Stress	CYP2A13	Human cytochrome P450
Stress	CYP2D6	Human mRNA for cytochrome P450 db1 variant b
Stress-apoptosis	BCL2A1	Homo sapiens BCL-2-related protein 1
Structural	CALB1	Homo sapiens calbindin 1

Structural	Elastin	Human elastin gene
Structural	KRT18	Human mRNA fragment for cytokeratin 18
Surface-Ig	IGM	Human gene for immunoglobulin mu
Surface-Ig	VH4	Human IgM heavy chain variable V-D-J region (VH4) gene
Surface-other	APP	Homo sapiens APP complete sequence
Surface-receptor	BDKRB1	Human bradykinin B1 receptor
Surface-receptor	TLR1	Human mRNA for KIAA0012 gene
Surface-receptor	5T4	Homo sapiens 5T4 oncofetal trophoblast glycoprotein
Surface-receptor	EFL-2	Homo sapiens EHK1 receptor tyrosine kinase ligand
Surface-receptor	EV12A	Homo sapiens ecotropic viral integration site 2A
Surface-receptor	FLT3	Homo sapiens fms-related tyrosine kinase 3
Surface-receptor	TNFSF10	Human tumor necrosis factor (ligand) superfamily, member 10
Surface-receptor	LTB	Human lymphotoxin beta
Surface-receptor	CDW52	Homo sapiens mRNA for CAMPATH-1
Surface-receptor	CLECSF2	Homo sapiens C-type lectin (activation-induced)
Surface-unknown	GliPR	Human glioma pathogenesis-related protein
Transport	LRP	Homo sapiens lrp mRNA
Transcription-RUNT	AML1	Human AML1 protein
Transcription-PAR-bZIP	TEF	Human hepatic leukemia factor
Transcription-FKH	FKHR	Homo sapiens forkhead protein
Transcription-suppressor	MN1	Homo sapiens chromosome 22q11.2 MDR region
Transcription-bHLH	ID1	Homo sapiens inhibitor of DNA binding 1
Transcription-bHLH	ID3	Homo sapiens HLH 1R21 mRNA for helix-loop-helix protein
Transcription-bHLH	EPAS1	Homo sapiens endothelial PAS domain protein 1
Transcription-bHLH	ID2	Homo sapiens inhibitor of DNA binding 2
Transcription-GATA	HGATA3	Homo sapiens GATA-binding protein 3
Transcription-HMG	hTcf-4	Homo sapiens mRNA for hTCF-4
Transcription-HOX	PHOX1	Human homeobox protein
Transcription-HOX	MEIS1	Homo sapiens MEIS protein
Transcription-slicing	RBP-MS	Homo sapiens RNA-binding protein gene with multiple slicing
Transcription-Translation	TCEA2	Homo sapiens transcription elongation factor A

Unknown	DIF2	IEX-1= radiation-inducible immediate-early gene
Unknown		Homo sapiens chromosome 17clone hRPC.906_A_24
Unknown		Homo sapiens chromosome 22q13 BAC clone CIT987SK-384D8
Unknown	A-362G6.1	Human chromosome 16 BAC clone CIT987SK-A-362G6
Unknown	LST1	Homo sapiens LST1 mRNA
Unknown	KIAA0125	Homo sapiens KIAA0125 gene product

Table 2. Genes Upregulated in Human HSCs from both Bone Marrow and Peripheral Blood

Classification	Name	Description
Hormone	AVP	Homo sapiens arginine vasopressin mRNA
Hormone		Corticotropin releasing hormone-binding protein
Enzyme	GUCY1A3	Homo sapiens guanylate cyclase 1, soluble, alpha 3
Enzyme	PPKCZ	Homo sapiens protein kinase C, zeta
Enzyme		Iduronate 2-sulfatase (Hunter syndrome)
Transcription factor	HLF	Human hepatic leukemia factor
Transcription factor	GATA3	Homo sapiens GATA-binding protein 3
Transcription	Evi1	Homo sapiens ecotropic viral integration site 1
Transcription	PMX1	Paired mesoderm homeo box 1
Transcription	MN1	Meningioma (disrupted in balanced translocation)
Secreted protein		Tetranectin (plasminogen-binding protein)
Secreted protein		H factor (complement)-like 1
Surface molecule		Transient receptor potential channel 1
Surface molecule	DLK1	Delta-like homolog (Drosophila)
Surface molecule	EphA3	Ephrin-A3
Surface molecule	TNFSF10	Human tumor necrosis factor (ligand) superfamily, member 10
Surface molecule		Interferon induced transmembrane protein
Surface molecule		Ecotropic viral integration site 2A
Surface molecule		Sortilin-related receptor, L (DLR class) A rep
Surface molecule		Major histocompatibility complex, class I, E
Surface molecule		KIAA0125 gene product

II. Definition

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by those of ordinary skill in the art to which this invention pertains. The following references provide one of skill with a general definition of many of the terms used in this invention: Singleton *et al.*, DICTIONARY OF MICROBIOLOGY AND MOLECULAR BIOLOGY (2d ed. 1994); THE CAMBRIDGE DICTIONARY OF SCIENCE AND TECHNOLOGY (Walker ed., 1988); and Hale & Marham, THE HARPER COLLINS DICTIONARY OF BIOLOGY (1991). In addition, the following definitions are provided to assist the reader in the practice of the invention.

The term "analog" is used herein to refer to a molecule that structurally resembles a reference molecule but which has been modified in a targeted and controlled manner, by replacing a specific substituent of the reference molecule with an alternate substituent. Compared to the reference molecule, an analog would be expected, by one skilled in the art, to exhibit the same, similar, or improved utility. Synthesis and screening of analogs, to identify variants of known compounds having improved traits (such as higher binding affinity for a target molecule) is an approach that is well known in pharmaceutical chemistry.

As used herein, "contacting" has its normal meaning and refers to combining two or more agents (e.g., polypeptides or small molecule compounds) or combining agents and cells (e.g., a polypeptide and a cell). Contacting can occur in vitro, e.g., combining two or more agents or combining a test agent and a cell or a cell lysate in a test tube or other container. Contacting can also occur in a cell or in situ, e.g., contacting two polypeptides in a cell by coexpression in the cell of recombinant polynucleotides encoding the two polypeptides, or in a cell lysate.

An "effective amount or dose" is an amount sufficient to effect beneficial or desired results. An effective amount may be administrated in one or more administrations. Determination of an effective amount is within the capability of those skilled in the art. Particularly preferred subjects of the invention in general include living mammals such as human, mice and rabbit, most preferred are humans. The administration of an HSC differentiation-inhibiting polypeptide, or a genetically modified cell comprising a polynucleotide sequence of the invention, may be by conventional means, for example, injection, oral administration, inhalation and others. Appropriate carries and diluents may be

included in the administration of the polypeptide or the modified cells. Samples including the modified cells and progeny thereof may be taken and tested to determine transduction efficiency.

The term "fragment" when used in connection with an amino acid sequence means a part of a reference sequence and having at least 10 amino acid residues, preferably 50 amino acids residues, even more preferably 100 amino acid residues and most preferably 200 amino acid residues which are substantially identical to the reference amino acid sequences. Where referring to a nucleotide sequence, the term means a nucleotide sequence including part of the reference sequence and comprising as few as at least 30, 50, 75, 80, 100 or more contiguous nucleotides, preferably at least 200, 300, 400, 500, 600, or more contiguous nucleotides, even more preferably at least 800, 1000, 1500, 2000 or more contiguous nucleotides that are identical to the reference sequence.

The term "functional equivalent" when referring to a polypeptide means a protein having a like function and like or improved specific activity, and a similar amino acid sequence. In some embodiments, a functionally equivalent is a variant in which one or more amino acid residues are substituted with conserved or non-conserved amino acid residues, or one in which one or more amino acid residues includes a substituent group. Conservative substitutions are the replacements, one for another, among the aliphatic amino acids Ala, Val, Leu and Ile; interchange of the hydroxl residues Ser and Thr; exchange of the acidic residues Asp and Glu; substitution between amide residues Asn and Gln; exchange of the basic residues Lys and Arg; and replacements among aromatic residues Phe and Tyr.

A "heterologous sequence" or a "heterologous nucleic acid," as used herein, is one that originates from a source foreign to the particular host cell, or, if from the same source, is modified from its original form. Thus, a heterologous gene in a host cell includes a gene that, although being endogenous to the particular host cell, has been modified. Modification of the heterologous sequence can occur, e.g., by treating the DNA with a restriction enzyme to generate a DNA fragment that is capable of being operably linked to the promoter. Techniques such as site-directed mutagenesis are also useful for modifying a heterologous nucleic acid.

The term "homologous" when referring to proteins and/or protein sequences indicates that they are derived, naturally or artificially, from a common ancestral protein or protein sequence. Similarly, nucleic acids and/or nucleic acid sequences are homologous

when they are derived, naturally or artificially, from a common ancestral nucleic acid or nucleic acid sequence. Homology is generally inferred from sequence similarity between two or more nucleic acids or proteins (or sequences thereof). The precise percentage of similarity between sequences that is useful in establishing homology varies with the nucleic acid and protein at issue, but as little as 25% sequence similarity is routinely used to establish homology. Higher levels of sequence similarity, e.g., 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95% or 99% or more can also be used to establish homology. Methods for determining sequence similarity percentages, e.g., BLASTP and BLASTN using default parameters, are well known and described in the art.

The terms "identical sequence" and "sequence identity" in the context of two nucleic acid sequences or amino acid sequences refer to the residues in the two sequences which are the same when aligned for maximum correspondence over a specified comparison window. A "comparison window", as used herein, refers to a segment of at least about 20 contiguous positions, usually about 50 to about 200, more usually about 100 to about 150 in which a sequence may be compared to a reference sequence of the same number of contiguous positions after the two sequences are aligned optimally. Methods of alignment of sequences for comparison are well-known in the art. Optimal alignment of sequences for comparison may be conducted by the local homology algorithm of Smith and Waterman (1981) Adv. Appl. Math. 2:482; by the alignment algorithm of Needleman and Wunsch (1970) J. Mol. Biol. 48:443; by the search for similarity method of Pearson and Lipman (1988) Proc. Nat. Acad. Sci U.S.A. 85:2444; by computerized implementations of these algorithms (including, but not limited to CLUSTAL in the PC/Gene program by Intelligentics, Mountain View, CA; and GAP, BESTFIT, BLAST, FASTA, or TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group (GCG), 575 Science Dr., Madison, Wis., U.S.A.). The CLUSTAL program is well described by Higgins and Sharp (1988) Gene 73:237-244; Higgins and Sharp (1989) CABIOS 5:151-153; Corpet et al. (1988) Nucleic Acids Res. 16:10881-10890; Huang et al (1992) Computer Applications in the Biosciences 8:155-165; and Pearson et al. (1994) Methods in Molecular Biology 24:307-331. Alignment is also often performed by inspection and manual alignment.

The term "isolated" means that the material is removed from its original environment (e.g., the natural environment if it is naturally occurring). For example, a naturally-occurring nucleic acid, polypeptide, or cell present in a living animal is not

isolated, but the same polynucleotide, polypeptide, or cell separated from some or all of the coexisting materials in the natural system, is isolated, even if subsequently reintroduced into the natural system. Such nucleic acids can be part of a vector and/or such nucleic acids or polypeptides could be part of a composition, and still be isolated in that such vector or composition is not part of its natural environment. When referring to a cell population, it means that homogeneous cells expressing a given set of molecular markers constitute at least 60%, preferably 75%, more preferably 90%, and most preferably 95% of the total number of cells in the population.

The terms "substantially identical" nucleic acid or amino acid sequences means that a nucleic acid or amino acid sequence comprises a sequence that has at least 90% sequence identity or more, preferably at least 95%, more preferably at least 98% and most preferably at least 99%, compared to a reference sequence using the programs described above (preferably BLAST) using standard parameters. For example, the BLASTN program (for nucleotide sequences) uses as defaults a wordlength (W) of 11, an expectation (E) of 10, M=5, N=-4, and a comparison of both strands. For amino acid sequences, the BLASTP program uses as defaults a wordlength (W) of 3, an expectation (E) of 10, and the BLOSUM62 scoring matrix (see Henikoff & Henikoff, Proc. Natl. Acad. Sci. USA 89:10915 (1989)). Percentage of sequence identity is determined by comparing two optimally aligned sequences over a comparison window, wherein the portion of the polynucleotide sequence in the comparison window may comprise additions or deletions (i.e., gaps) as compared to the reference sequence (which does not comprise additions or deletions) for optimal alignment of the two sequences. The percentage is calculated by determining the number of positions at which the identical nucleic acid base or amino acid residue occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the window of comparison and multiplying the result by 100 to yield the percentage of sequence identity. Preferably, the substantial identity exists over a region of the sequences that is at least about 50 residues in length, more preferably over a region of at least about 100 residues, and most preferably the sequences are substantially identical over at least about 150 residues. In a most preferred embodiment, the sequences are substantially identical over the entire length of the coding regions.

The terms "nucleic acid" and "polynucleotide" refer to a deoxyribonucleotide or ribonucleotide polymer in either single- or double-stranded form, and unless otherwise

limited, encompasses known analogues of natural nucleotides that hybridize to nucleic acids in manner similar to naturally occurring nucleotides. A "polynucleotide sequence" is a nucleic acid (which is a polymer of nucleotides (A,C,T,U,G, etc. or naturally occurring or artificial nucleotide analogues) or a character string representing a nucleic acid, depending on context. Either the given nucleic acid or the complementary nucleic acid can be determined from any specified polynucleotide sequence.

The term "operably linked" refers to a functional relationship between two or more polynucleotide (e.g., DNA) segments. Typically, it refers to the functional relationship of a transcriptional regulatory sequence to a transcribed sequence. For example, a promoter or enhancer sequence is operably linked to a coding sequence if it stimulates or modulates the transcription of the coding sequence in an appropriate host cell or other expression system. Generally, promoter transcriptional regulatory sequences that are operably linked to a transcribed sequence are physically contiguous to the transcribed sequence, i.e., they are cis-acting. However, some transcriptional regulatory sequences, such as enhancers, need not be physically contiguous or located in close proximity to the coding sequences whose transcription they enhance. A polylinker provides a convenient location for inserting coding sequences so the genes are operably linked to the promoter. Polylinkers are polynucleotide sequences that comprise a series of three or more closely spaced restriction endonuclease recognition sequences.

As used herein the term "overexpression" refers to expression of a polypeptide brought about by genetic modification of a host cell with a nucleic acid sequence encoding the polypeptide. Overexpression may take place in cells normally lacking expression of the polypeptide (e.g., an HSC differentiation-inhibiting polypeptide). It can also occur in cells with endogenous expression of the polypeptide. While overexpression may take place in any cell type, preferred host cells for overexpressing an HSC differentiation-inhibiting polypeptide are hematopoietic stem cells.

The terms "polypeptide" and "protein" are used interchangeably herein, and refer to a polymer of amino acid residues, e.g., as typically found in proteins in nature. A "mature protein" is a protein which is full-length and which, optionally, includes glycosylation or other modifications typical for the protein in a given cell membrane.

A "variant" of a molecule such as an HSC differentiation-inhibiting polypeptide is meant to refer to a molecule substantially similar in structure and biological

activity to either the entire molecule, or to a fragment thereof. Thus, provided that two molecules possess a similar activity, they are considered variants as that term is used herein even if the composition or secondary, tertiary, or quaternary structure of one of the molecules is not identical to that found in the other, or if the sequence of amino acid residues is not identical. In some embodiments, a variant differs in amino acid sequence from a reference polypeptide by one or more substitutions, additions, deletions, truncations which may be present in any combination. Among preferred variants are those that vary from a reference polypeptide by conservative amino acid substitutions. Such substitutions are those that substitute a given amino acid by another amino acid of like characters. The following non-limiting list of amino acids are considered conservative replacements: a) alanine, serine, and threonine; b) glutamic acid and asparatic acid; c) asparagine and glutamine d) arginine and lysine; e) isoleucine, leucine, methionine and valine and f) phenylalaine, tyrosine and tryptophan. Most highly preferred are variants that retain the same biological function and activity as the reference polypeptide from which it varies.

III. Promoting HSC Expansion by Inhibiting Differentiation

In addition to novel markers and methods for isolating HSCs, the invention also provides methods for inhibiting or blocking differentiation of mammalian hematopoietic stem cells, thereby promoting expansion of the stem cells. A number of the novel HSC marker genes identified in the present invention can inhibit or block HSC differentiation. Examples of such differentiation-inhibiting genes are shown in Tables 1 and 2 (for human HSC) and Tables 3 and 4 (for mouse HSC). For example, as described in the Examples below, human stem cells overexpressing GATA-binding protein 3 slows differentiation of the cells. HSCs overexpressing ID3 increased colony forming cells, indicating enhanced HSC activity as compared to a control. These differentiation-inhibiting molecules can be used in the present invention to inhibit HSC differentiation and thereby promoting expansion in vitro. They can also be used in vivo to increase the effective dose of engrafted HSCs in a subject.

The term HSC differentiation-inhibiting molecules (polynucleotides and the encoded polypeptides) include the molecules shown in Tables 1-4 that inhibit or slow HSC differentiation. Polynucleotides with substantial sequence identity are also encompassed. In addition, they also include variants, analogs, fragments, or functional derivatives of the HSC

differentiation-inhibiting molecules shown in Tables 1-4. These differentiation-inhibiting molecules can be obtained from any species. Preferably, they are from mammalian species including human, mouse, and chicken. The HSC differentiation-inhibiting molecules can also be from any source whether natural, synthetic or recombinant.

Differentiation is defined as the restriction of the potential of a cell to self-renew and is normally associated with a change in the functional capacity of the cell. The term "inhibiting" or "blocking" differentiation is used broadly in the context of this invention and includes not only the prevention of differentiation but also encompasses altering or slowing differentiation process of a cell. Differentiation of a stem cell can be determined by methods well known in the art and these include analysis for surface markers associated with cells of a defined differentiated state.

An HSC differentiation-inhibiting polypeptide of the present invention encodes an HSC differentiation-inhibiting polypeptide that blocks or slows down differentiation of the HSC cells (e.g., as listed in Tables 1-4). As shown in the Tables, these molecules include hormones, secreted proteins, or growth factors. These molecules also include transcription factors. One or more of these HSC differentiation-inhibiting polypeptides, or fragments thereof, can be applied to HSC cells in vitro, e.g., in a cell culture. These cells can be cultured and grown as described herein or other methods well known in the art. The appropriate amount of these differentiation-inhibiting polypeptides to be used in the cultures can be easily determined in accordance with stem cell culturing procedures described herein or knowledge well known in the art. By culturing the HSC in the presence of these molecules, differentiation of the cells can be inhibited or slowed, resulting in enhanced growth of engraftable HSCs.

In addition to promoting HSC expansion in vitro, the HSC differentiation-inhibiting polypeptides of the invention can also be administered directly to a subject to promote in vivo growth of HSCs. For example, a subject engrafted with bone marrow or a population of HSCs can also be administered an effective amount of an HSC differentiation-inhibiting polypeptide or fragment thereof (e.g., the secreted proteins or growth factors shown in Table 1 and Tables 3-4). The polypeptide can be administered to the subject prior to, concurrently with, or subsequent to transplantation of the bone marrow or HSCs. Preferably, the polypeptide and the HSCs are administered to the subject simultaneously.

Other than using a differentiation-inhibiting polypeptide, inhibition of HSC differentiation can also be achieved using an HSC differentiation-inhibiting polynucleotide to genetically modify HSCs. HSC differentiation-inhibiting polynucleotides suitable for these methods include some of the genes upregulated in HSCs (as shown in Tables 1 and 3). They encode HSC differentiation-inhibiting polypeptides that block or slow down differentiation of the HSC cells. Some of these methods require first isolation of a population of hematopoietic cells, e.g., a population of $CD34^+Thy^+$ human cells or $CD34^-$ $CD38^+$ mouse cells as described above, from a source of such cells. An HSC differentiation-inhibiting polynucleotide of the invention can then be introduced into the cells whereby the cells are genetically modified.

Once the cells are genetically modified, they are cultured in the presence of at least one cytokine in an amount sufficient to support growth of the modified cells. The modified cells are then selected wherein the encoded polypeptide is overexpressed and differentiation is blocked. The genetically modified cells thus obtained may be used immediately (e.g., in transplant), cultured and expanded in vitro, or stored for later uses. The modified HSCs may be stored by methods well known in the art, e.g., frozen in liquid nitrogen.

Genetic modification as used herein encompasses any genetic modification method of introduction of an exogenous or foreign gene into mammalian cells (particularly human stem cell and hematopoietic cells). The term includes but is not limited to transduction (viral mediated transfer of host DNA from a host or donor to a recipient, either in vitro or in vivo), transfection (transformation of cells with isolated viral DNA genomes), liposome mediated transfer, electroporation, calcium phosphate transfection or coprecipitation and others. Methods of transduction include direct co-culture of cells with producer cells (Bregni et al., Blood 80:1418-1422, 1992) or culturing with viral supernatant alone with or without appropriate growth factors and polycations (Xu et al., Exp. Hemat. 22:223-230, 1994).

Various in vitro and in vivo assays are well known in the art for the measurement of the functional compositions of hematopoietic cell populations. See, e.g., Quesenberry et al. eds., Stem Cell Biology and Gene Therapy, Wiley-Liss Inc. 1998--Chapter 5, Hematopoietic Stem cells: Proliferation, Purification and Clinical Applications, pgs 133-160. Other examples of suitable assays are also known in the art. For example, the

long term culture-initiating cell (LTCIC) assay involves culturing a cell population on stromal cell monolayers for approximately 5 weeks and then testing in a 2 week semisolid media culture for the frequency of clonogenic cells retained (Sutherland et al., Blood 74:1563 (1989)). The Colony Forming Cells (CFC) assay or Colony-Forming Unit Culture (CFUC) assay involves use of cell count as the number of colony-forming units per unit volume or area of a sample. The assay is used to measure clonal growth of quickly maturing progenitors in semi-solid media supplemented with serum and growth factors. Depending on the growth factors used to stimulate growth mature and/or primitive progenitors may be determined. Cobblestone area forming colony (CAFC) assays measure clonal proliferation of long-lived progenitors supported by stromal cell monolayers and growth factor/serum supplemented media. On the appropriate stromal monolayers, cells pluripotent for myeloid and lymphoid lineages may be determined. (Young et al., Blood 88:1619, 1996). SCID-hu bone assays measure the proliferation and multilineage differentiation of cells with bone marrow repopulating activity. These cells are likely to contribute to durable engraftment in clinical transplantation. SCID-hu thymus assays measure the proliferation and differentiation in thymocytes. Both bone marrow repopulating and more mature T-lineage progenitors may be measured.

A polynucleotide encoding an HSC differentiation-inhibiting molecule is typically introduced to a host cell in a vector. The vector typically includes the necessary elements for the transcription and translation of the inserted coding sequence. Methods used to construct such vectors are well known in the art. For example, techniques for constructing suitable expression vectors are described in detail in Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Press, N.Y. (3rd Ed., 2000); and Ausubel et al., *Current Protocols in Molecular Biology*, John Wiley & Sons, Inc., New York (1999).

Vectors may include but are not limited to viral vectors, such as baculovirus, retroviruses, adenoviruses, adeno-associated viruses, and herpes simplex viruses; bacteriophages; cosmids; plasmid vectors; synthetic vectors; and other recombination vehicles typically used in the art. Vectors containing both a promoter and a cloning site into which a polynucleotide can be operatively linked are well known in the art. Such vectors are capable of transcribing RNA in vitro or in vivo, and are commercially available from sources such as Stratagene (La Jolla, Calif.) and Promega Biotech (Madison, Wis.). Specific

examples include, pSG, pSV2CAT, pXt1 from Stratagene; and pMSG, pSVL, pBPV and pSVK3 from Pharmacia.

Preferred vectors include retroviral vectors (see, Coffin et al., "Retroviruses", Chapter 9 pp; 437-473, Cold Springs Harbor Laboratory Press, 1997). Vectors useful in the invention can be produced recombinantly by procedures well known in the art. For example, WO94/29438, WO97/21824 and WO97/21825 describe the construction of retroviral packaging plasmids and packing cell lines. Exemplary vectors include the pCMV mammalian expression vectors, such as pCMV6b and pCMV6c (Chiron Corp.), pSFFV-Neo, and pBluescript-Sk+. Non-limiting examples of useful retroviral vectors are those derived from murine, avian or primate retroviruses. Common retroviral vectors include those based on the Moloney murine leukemia virus (MoMLV-vector). Other MoMLV derived vectors include, Lmily, LINGFER, MINGFR and MINT (Chang et al., Blood 92:1-11, 1998). Additional vectors include those based on Gibbon ape leukemia virus (GALV) and Moloney murine sacroma virus (MoMSV) and spleen focus forming virus (SFFV). Vectors derived from the murine stem cell virus (MESV) include MESV-MiLy (Agarwal et al., J. of Virology, 72:3720-3728, 1998). Retroviral vectors also include vectors based on lentiviruses, and non-limiting examples include vectors based on human immunodeficiency virus (HIV-1 and HIV-2).

In producing retroviral vector constructs, the viral gag, pol and env sequences can be removed from the virus, creating room for insertion of foreign DNA sequences. Genes encoded by foreign DNA are usually expressed under the control a strong viral promoter in the long terminal repeat (LTR). Selection of appropriate control regulatory sequences is dependent on the host cell used and selection is within the skill of one in the art. Numerous promoters are known in addition to the promoter of the LTR. Non-limiting examples include the phage lambda PL promoter, the human cytomegalovirus (CMV) immediate early promoter; the U3 region promoter of the Moloney Murine Sarcoma Virus (MMSV), Rous Sacroma Virus (RSV), or Spleen Focus Forming Virus (SFFV); Granzyme A promoter; Granzyme B promoter, CD34 promoter; and the CD8 promoter. Additionally inducible or multiple control elements may be used.

Such a construct can be packed into viral particles efficiently if the gag, pol and env functions are provided in trans by a packing cell line. Therefore, when the vector construct is introduced into the packaging cell, the gag-pol and env proteins produced by the

cell, assemble with the vector RNA to produce infectious virons that are secreted into the culture medium. The virus thus produced can infect and integrate into the DNA of the target cell, but does not produce infectious viral particles since it is lacking essential packaging sequences. Most of the packing cell lines currently in use have been transfected with separate plasmids, each containing one of the necessary coding sequences, so that multiple recombination events are necessary before a replication competent virus can be produced. Alternatively the packaging cell line harbors a provirus. The provirus has been crippled so that although it may produce all the proteins required to assemble infectious viruses, its own RNA cannot be packaged into virus. RNA produced from the recombinant virus is packaged instead. Therefore, the virus stock released from the packaging cells contains only recombinant virus. Non-limiting examples of retroviral packaging lines include PA12, PA317, PE501, PG13, PSI.CRIP, RD114, GP7C-tTA-G10, ProPak-A (PPA-6), and PT67. Reference is made to Miller et al., Mol. Cell Biol. 6:2895, 1986; Miller et al., Biotechniques 7:980, 1989; Danos et al., Proc. Natl. Acad. Sci. USA 85:6460, 1988; Pear et al., Proc. Natl. Acad. Sci. USA 90:8392-8396, 1993; and Finer et al., Blood 83:43-50, 1994.

Other suitable vectors include adenoviral vectors (see, Frey et al., Blood 91:2781, 1998; and WO 95/27071) and adeno-associated viral vectors. These vectors are all well know in the art, e.g., as described in Chatterjee et al., Current Topics in Microbiol. And Immunol., 218:61-73, 1996; Stem cell Biology and Gene Therapy, eds. Quesenberry et al., John Wiley & Sons, 1998; and U.S. Pat. Nos. 5,693,531 and 5,691,176. The use of adenovirus-derived vectors may be advantageous under certain situation because they are not capable of infecting non-dividing cells. Unlike retroviral DNA, the adenoviral DNA is not integrated into the genome of the target cell. Further, the capacity to carry foreign DNA is much larger in adenoviral vectors than retroviral vectors. The adeno-associated viral vectors are another useful delivery system. The DNA of this virus may be integrated into non-dividing cells, and a number of polynucleotides have been successful introduced into different cell types using adeno-associated viral vectors.

In some embodiments, the construct or vector will include two or more heterologous polynucleotide sequences; a) the nucleic acid sequence encoding an HSC differentiation-inhibiting polypeptide of the invention, and b) one or more additional nucleic acid sequence. Preferably the additional nucleic acid sequence is a polynucleotide which

encodes a selective marker, a structural gene, a therapeutic gene, a ribozyme, or an antisense sequence.

A selective marker may be included in the construct or vector for the purposes of monitoring successful genetic modification and for selection of cells into which DNA has been integrated. Non-limiting examples include drug resistance markers, such as G148 or hygromycin. Additionally negative selection may be used, for example wherein the marker is the HSV-tk gene. This gene will make the cells sensitive to agents such as acyclovir and gancyclovir. Selection may also be made by using a cell surface marker, for example, to select overexpression of an HSC differentiation-inhibiting polypeptide by fluorescence activated cell sorting (FACS). The NeoR (neomycin/G148 resistance) gene is commonly used but any convenient marker gene may be used whose gene sequences are not already present in the target cell can be used. Further non-limiting examples include low-affinity Nerve Growth Factor (NGFR), enhanced fluorescent green protein (EFGP), dihydrofolate reductase gene (DHFR) the bacterial hisD gene, murine CD24 (HSA), murine CD8a(lyt), bacterial genes which confer resistance to puromycin or phleomycin, and beta.-glactosidase.

The additional polynucleotide sequence(s) may be introduced into the host cell on the same vector as the polynucleotide sequence encoding the polypeptides of the invention or the additional polynucleotide sequence may be introduced into the host cells on a second vector. In a preferred embodiment, a selective marker will be included on the same vector as the HSC differentiation-inhibiting polynucleotide.

Typically, the host cells for expressing the HSC differentiation-inhibiting polynucleotide are mammalian stem cells, e.g., HSCs from humans, mice, monkeys, farm animals, sport animals, pets, and other laboratory rodents and animals. These cells can be obtained, cultured, and manipulated as described above and in Potten C. S. ed., Stem Cells, Academic Press, 1997; Stem Cell Biology and Gene Therapy, eds. Quesenberry et al., John Wiley & Sons Inc., 1998; and Gage et al., Ann. Rev. Neurosci. 18:159-192, 1995.

IV. Novel Molecular Markers for Isolating and Enriching HSCs

As detailed in the Examples below, the present inventor identified a number of genes that are differentially expressed in human and mouse HSCs. These genes, which can play a role in regulating hematopoiesis as well as activities of HSCs and progenitor cells,

are suitable as markers for selecting and enriching HSCs from diverse populations of cells. As exemplified in Tables 1-4, these HSC markers include transmembrane proteins (e.g., receptors), growth factor, transcription factors, as well as other proteins with diverse cellular and biochemical functions.

Employing these novel HSC markers, the present invention provides methods for isolating stem cells from any vertebrate, particularly mammalian, species. In general, one or more of the novel markers can be targeted in the methods. Selection with these markers can be performed alone with a crude population of cells (e.g., bone marrow). The selection scheme can also be used in combination with other selection and purification procedures, e.g., to further select HSCs from cells already enriched for other known HSC surface markers.

In some embodiments, the novel markers for selecting and enriching HSCs are cell surface markers. As described in the Examples, a number of the genes upregulated in the human and mouse HSCs encode transmembrane proteins (see also Tables 2 and 7). These proteins provide novel surface markers for isolating HSCs from or enumerating HSCs in a population of diverse cells (e.g., bone marrow). These methods are useful for isolating stem cells from primates, e.g. human, monkeys, gorillas, domestic animals, bovine, equine, ovine, porcine, and etc. Isolation of HSCs bearing these novel markers can be performed with the same procedures disclosed herein for the other phenotypic markers.

In some embodiments, selection of the novel HSC markers utilizes antibodies that recognize the novel HSC markers. This includes preparing an antibody to a novel HSC marker (e.g., a surface marker) of the invention and purifying the antibody. By exposing a population of hematopoietic cells or crude cells to the antibody and allowing the exposed cells to bind with the antibody, cells bearing the novel HSC marker can be isolated. Techniques including antibody preparation and purification are well known and routinely practiced in the art. See, e.g., Harlow and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Press (1998). Such antibodies encompass any antibody or fragment thereof either native or recombinant, synthetic or naturally derived, which retains sufficient specificity to bind specifically to an HSC marker. They may be monoclonal or polyclonal, and can be produced using the novel HSC marker protein or a fragment or variant thereof. In addition, antibodies that recognize some of these marker proteins may also be obtained commercially.

When combined with other selection procedures, the particular order by which hematopoietic cells are separated from other cells is not critical to this invention. When a genetically modified HSC cell is to be selected (as detailed above), the specific cell types may be separated either prior to genetic modification or after genetic modification. In some methods, crude cell samples are initially separated by markers indicating unwanted cells, then with a negative selection, followed by separations for markers or marker levels indicating that the cells belong to the stem cell population, and finally positive selection with novel markers of the present invention. In some other methods, following the initial crude separation, the cells can be directly subject to enrichment for at least one of the novel HSC markers.

For example, an initial crude cell population can be first purified to remove major cell families from the bone marrow or other hematopoietic cell source. A negative selection can then be carried out by targeting some of the cell surface antigens (e.g., Lin, CD34 for mouse HSCs). A further positive selection can be performed to isolate a cell population with specific stem cell markers (e.g., CD34 and Thy for human HSC, and c-kit, Sca-1, or CD38 for mouse HSC). Thereafter, additional selections can be carried out using one or more of the novel HSC surface markers disclosed herein.

The starting cell populations for selecting and enriching HSC can be obtained from bone marrow or other hematopoietic source. Stem cells and progenitor cells from bone marrow constitute only a small percentage (e.g., about 0.01 to about 0.1%) of the bone marrow cells. Bone marrow cells may be obtained from a source of bone marrow, e.g. tibiae, femora, spine, fetal liver, and other bone cavities. Other sources of hematopoietic stem cells include embryonic yolk sac, fetal live, fetal and adult spleen, and blood including adult peripheral blood and umbilical cord blood (To et al., Blood 89:2233-2258, 1997).

Procedures for isolation of bone marrow are well known in the art. For example, an appropriate solution may be used to flush the bone. For example, the solution can be a balanced salt solution conveniently supplemented with fetal calf serum or other naturally occurring factors. These components can be present in conjunction with an acceptable buffer at low concentration, generally from about 5 to 25 mM. Convenient buffers include but are not limited to HEPES, phosphate and lactate buffers. Bone marrow can also be aspirated from the bone in accordance with other conventional techniques well known in the art.

As indicated above, to isolate the HSC cells, a relatively crude separation can be initially used to remove major cell families from the bone marrow or other hematopoietic cell source. Various techniques may be employed to separate the cells to initially remove cells of dedicated lineage. These include physical separation, magnetic separation using antibody-coated magnetic beads, affinity chromatography, and cytotoxic agents joined to a monoclonal antibody or used in conjunction with a monoclonal antibody. Also included is the use of fluorescence activated cell sorters (FACS) wherein the cells can be separated on the basis of the level of staining of the particular antigens. These techniques are well known to those of ordinary skill in the art and are described in various references including U.S. Pat. Nos. 5,061,620; 5,409,8213; 5,677,136; and 5,750,397; and Yau et al., Exp. Hematol. 18:219-222, 1990).

Monoclonal antibodies are particularly useful for this initial separation procedure. The antibodies may be attached to a solid support to allow for separation. In some methods, magnetic bead separations are used to attach the antibodies. Conjugating the antibodies with markers such as magnetic beads, e.g., using biotin-avidin link, allows for direct separation of bound cells from the unbound cells. Antibodies (e.g., monoclonal antibodies) directed to the various surface markers of these differentiated cells can be obtained commercially or prepared using methods routinely practiced in the art.

To select HSCs, this initial separation allows removal of large numbers of cells of the hematopoietic system of various lineages, such as thymocytes, T-cells, pre-B cells, B-cells, granulocytes, myelomonocytic cells, and platelets. Cells that can be separated in this stage also include other minor cell populations, e.g., megakaryocytes, mast cells, eosinophils and basophils. Generally, at least about 70%, usually 80% or more of the total hematopoietic cells will be removed. Since there will be positive selection at the later selection steps, it is not essential to remove at the initial stage every dedicated cell class, such as the minor population members, the platelets, and erythrocytes. However, it is preferable that there be positive selection for all of the cell lineages, so that in the final positive selection the number of dedicated cells present is minimized.

Phenotypes of surface antigen of the dedicated lineage cells are known in the art. For example, CD34 is expressed on most immature T-cells also called thymocytes, and these cells lack cell surface expression of CD1, CD2, CD3, CD4, and CD8 antigens. CD45RA is a useful T-cell marker. The best known T-cell marker is the T-cell receptor

(TCR). There are presently two defined types of TCRs, TCR-2 (consisting of α and β polypeptides) and TCR-1 (consisting of δ and γ polypeptides). B cells may be selected, for example, by expression of CD19 and CD20. Myeloid cells may be selected, for example, by expression of CD14, CD15, and CD16. NK cells may be selected based on expression of CD56 and CD16. Erythrocytes may be identified by expression of glycophorin A. Compositions enriched for progenitor cells capable of differentiation into myeloid cells, dendritic cells, or lymphoid cells also include the phenotypes $CD45RA^+$ $CD34^+$ $Thy1^+$ and $CD45RA^+$ $CD10^+$ Lin^- $CD34^+$. Other useful markers for various cell types are also known in the art.

The separation techniques employed should maximize the retention of viability of the fraction to be collected. For the initial separations, various techniques of differing efficacy may be employed. The particular technique employed will depend upon efficiency of separation, cytotoxicity of the methodology, ease and speed of performance, and necessity for sophisticated equipment and/or technical skill. Procedures for separation may include magnetic separation, using antibody-coated magnetic beads, affinity chromatography, cytotoxic agents joined to a monoclonal antibody or used in conjunction with a monoclonal antibody, e.g. complement and cytotoxins, and "panning" with antibody attached to a solid matrix, e.g. plate. Techniques providing accurate separation include fluorescence activated cell sorters, which can have varying degrees of sophistication, e.g. a plurality of color channels, low angle and obtuse light scattering detecting channels, and impedance channels.

Following the initial coarse selection, positive and/or negative selection using various other known stem cell markers as well as the novel HSC markers disclosed herein can be followed. In some methods, human HSCs are isolated using markers such as $CD34^+$ and Thy^+ as discussed in the Examples below. In some methods, human HSCs are selected for a phenotype of $CD34^+$ $Thy1^+$ Lin^-. Other examples of enriched phenotypes include: $CD2^-$, $CD3^-$, $CD4^-$, $CD8^-$, $CD10^-$, $CD14^-$, $CD15^-$, $CD19^-$, $CD20^-$, $CD33^-$, $CD34^-$, $CD38^{lo/-}$, $CD45RA^-$, CD $59^{+/-}$, $CD71^-$, $CDW109^+$, $glycophorin^-$, $AC133^+$, $HLA\text{-}DR^{+/-}$, $c\text{-}kit^+$, and EM^+. Lin^- refers to a cell population selected on the basis of lack of expression of at least one lineage specific marker, for example CD2, CD3, CD14, and CD56. The combination of expression markers used to isolate and define an enriched HSC population may vary depending on various factors and may vary as other expression markers become available.

Similarly, mouse HSCs can be selected for one or more of the known markers such as Lin^-, c-kit^+, Sca-1^+, $CD38^+$, and $CD34^-$ (see Example 3). In other methods, murine HSCs with similar properties to the human $CD34^+$ Thy-1^+ Lin^- may be identified by kit^+ Thy-1.1^{lo} $Lin^{-/lo}$ Sca-1^+ (KTLS). Other phenotypes are well known, e.g., as described in US Patent No. 6,451,558. When CD34 expression is combined with selection for Thy-1, a composition comprising approximately fewer than 5% lineage committed cells can be isolated (U.S. Pat. No. 5,061,620).

Once the cells are harvested and optionally separated, the cells are cultured in a suitable medium comprising a combination of growth factors that are sufficient to maintain growth. The term culturing refers to the propagation of cells on or in media of various kinds. It is understood that the descendants of a cell grown in culture may not be completely identical (either morphologically, genetically or phenotypically) to the parent cell. Methods for culturing stem cells and hematopoietic cells are well known to those skilled in the art. Any suitable culture container may be used, and these are readily available from commercial vendors. The seeding level is not critical, and it will depend on the type of cells used. In general, the seeding level will be at least 10 cells per ml, more usually at least about 100 cells per ml and generally not more than 10^6 cells per ml.

Various culture media can be used and non-limiting examples include Iscove's modified Dulbecco's medium (IMDM), X-vivo 15 and RPMI-1640. These are commercially available from various vendors. The formulations may be supplemented with a variety of different nutrients, growth factors, such as cytokines and the like. In general, the term cytokine refers to any one of the numerous factors that exert a variety of effects on cells, such as inducing growth and proliferation. The cytokines may be human in origin or may be derived from other species when active on the cells of interest. Included within the scope of the definition are molecules having similar biological activity to wild type or purified cytokines, for example produced by recombinant means, and molecules which bind to a cytokine factor receptor and which elicit a similar cellular response as the native cytokine factor.

The medium can be serum free or supplemented with suitable amounts of serum such as fetal calf serum, autologous serum or plasma. If cells or cellular products are to be used in humans, the medium will preferably be serum free or supplemented with

autologous serum or plasma (see, e.g., Lansdorp et al., J. Exp. Med. 175:1501, 1992; and Petzer et al., PNAS 93:1470, 1996).

Examples of compounds that can be used to supplement the culture medium are thrombopoietin (TPO), Flt3 ligand (FL), c-kit ligand (KL, also known as stem cell factor, SCF, or Stl), Interleukin (e.g., IL-1, IL-2, IL-3, IL-6, soluble IL-6 receptor, IL-11, and IL-12), granulocyte-colony stimulating factor (G-CSF), granulocyte macrophage-colony stimulating factor (GM-CSF), leukemia inhibitory factor (LIF), MIP-1α, and erythropoietin (EPO). These compounds may be used alone or in any combination. When murine stem cells are cultured, a preferred non-limiting medium includes mIL-3, mIL-6 and mSCF.

Concentration range of these compounds to be used in cultures can be determined according to knowledge well known in the art. For example, a general preferred range of TPO is from about 0.1 ng/mL to about 5000 μg/mL, more preferred is from about 1.0 ng/mL to about 1000 ng/mL, even more preferred from about 5.0 ng/mL to about 300 ng/mL. A preferred concentration range for each of FL and KL is from about 0.1 ng/mL to about 1000 ng/mL, more preferred is from about 1.0 ng/mL to about 500 ng/mL. IL-6 is a preferred factor to be included in the culture, and a preferred concentration range is from about 0.1 ng/mL to about 500 ng/mL, and more preferred from about 1.0 ng/mL to about 100 ng/mL. Hyper IL-6, a covalent complex of IL-6 and IL-6 receptor may also be used in the culture.

Other molecules can also be added to the culture media, for instance, adhesion molecules, such as fibronection or RetroNectin™ (commercially produced by Takara Shuzo Co., Otsu Shigi, Japan). Fibronectin is a glycoprotein that is found throughout the body, and its concentration is particularly high in connective tissues where it forms a complex with collagen.

V. Therapeutic Applications

HSC's are the active component in bone marrow transplantation (BMT). The use of purified HSCs transplant as opposed to bone marrow provides the advantage that transplant of harmful non-HSC cells in the bone marrow is avoided. In the autologous cancer or autoimmune setting, the use of purified HSCs minimizes the possibility of giving tumor or diseased cells back to the patient along with the bone marrow. In allogenic transplantion, using high doses of HSCs overcomes rejection by the recipient immune

system. Thus, expansion of HSCs would make autologous and allogeneic HSC transplantation safer and more effective.

The present invention provides methods for inhibiting HSC differentiation and promoting HSC expansion in vivo in a subject, e.g., a human subject engrafted with HSCs. Using HSC differentiation-inhibiting molecules identified in the present invention, these methods allow expansion of non-differentiated stem cells and increase the dose of HSCs either *ex vivo* or *in vivo*, thereby potentially allowing more rapid engraftment. The HSC differentiation-inhibiting molecules can be expressed in the engrafted HSCs. It can also be separately provided to the subject receiving the HSC graft, e.g., expressed from a vector introduced into the subject. In addition, the HSC differentiation-inhibiting molecules can also be administered to the subject as an expressed polypeptide, e.g., a growth factor. As a result, differentiation of the cells is blocked or slowed down, resulting in expansion of non-differentiated stem cells.

Some methods of the invention provide *ex vivo* gene therapy for transplanting genetically modified HSCs cells into a subject. For example, vectors expressing an HSC differentiation-inhibiting polypeptide can be delivered to HSCs explanted from an individual subject, followed by reimplantation of the cells into a subject, usually after selection for cells that have incorporated the vector. Procedures for modifying host cells with an HSC differentiation-inhibiting polynucleotide (e.g., GATA3) are described above. In addition, *ex vivo* cell transfection for diagnostics, research, or for gene therapy (*e.g.*, via re-infusion of the transfected cells into the host organism) is well known in the art. For a review of gene therapy procedures, *see* Anderson, *Science* 256: 808-813, 1992; Nabel & Felgner, TIBTECH 11: 211-217, 1993; Mitani & Caskey, TIBTECH 11: 162-166, 1993; Mulligan, *Science* 260: 926-932, 1993; Dillon, TIBTECH 11: 167-175, 1993; Miller, *Nature* 357: 455-460, 1992; Van Brunt, *Biotechnology* 6: 1149-1154, 1998; Vigne, *Restorative Neurology and Neuroscience* 8: 35-36, 1995; Kremer & Perricaudet, *British Medical Bulletin* 51: 31-44, 1995; Haddada *et al.*, in *Current Topics in Microbiology and Immunology* (Doerfler & Böhm eds., 1995); and Yu *et al.*, *Gene Therapy* 1: 13-26, 1994).

For therapeutic applications, the genetically modified HSC cells are maintained for a period of time sufficient for overexpression of HSC differentiation-inhibiting polypeptide. A suitable time period will depend inter alia upon cell type used and is readily determined by one skilled in the art. In general, genetically modified cells of the

invention may overexpress HSC differentiation-inhibiting polypeptide for the lifetime of the host cell. Preferably, for hematopoietic cells the time period will be in the range of 1 to 45 days, more preferably in the range of 1 to 30 days, even more preferably in the range of 1 to 20 days, still more preferably in the range of 1 to 10 days, and most preferably in the range of 1 to 5 days.

Other than *ex vivo* gene therapy, vectors expressing an HSC differentiation-inhibiting polypeptide can also be delivered *in vivo*. This is carried out by administering to an individual subject the expression vector, typically by systemic administration (*e.g.*, intravenous, intraperitoneal, intramuscular, subdermal, or intracranial infusion) or topical application. Methods for *in vivo* gene therapy are also well known in the art, e.g., as described in the literatures noted above.

As described above, other than gene therapy, therapeutic expansion of HSCs in a subject can also be achieved by directly applying an HSC differentiation-inhibiting polypeptide (or its fragment or functional derivative) to a subject. The subject can be simultaneously engrafted with HSCs. The subject can also be one that has not been subject to HSC transplant. Typically, in such applications, the HSC differentiation-inhibiting polypeptide (e.g., GATA3) is administered to the subject in a pharmaceutical composition. The pharmaceutical compositions typically comprise at least one active ingredient together with one or more acceptable carriers thereof. Suitable carriers for preparing the pharmaceutical compositions, appropriate dosages, and suitable routes of administration of the compositions can all be readily determined by following methods well known in the art. See, e.g., Gilman et al., eds., Goodman and Gilman's: The Pharmacological Bases of Therapeutics , 8th ed., Pergamon Press, 1990; Remington: The Science and Practice of Pharmacy, Mack Publishing Co., 20th ed., 2000; Avis et al., eds., Pharmaceutical Dosage Forms: Parenteral Medications, published by Marcel Dekker, Inc., N.Y., 1993; and Lieberman et al., eds., Pharmaceutical Dosage Forms: Tablets, published by Marcel Dekker, Inc., N.Y., 1990.

EXAMPLES

The following examples are provided to illustrate, but not to limit the present invention.

Example 1. Genes Upregulated in Human HSCs

This Example describes RNA profiling of human hematopoietic stem cells and characterization of genes upregulated in the HSCs. All procedures and assays employed herein to study the human HSCs have been described in the art, e.g., as noted above.

$CD34^+$ cells were first isolated from blood of six normal human donors using magnetic beads. Flow activated cell sorting (FACS) was then used to purify $CD34^+Thy^+$ (stem enriched) and $CD34^+Thy^-$ (stem depleted) cell populations. The two populations of cells (total 12 samples, 6 $CD34^+Thy^+$ and 6 $CD34^+Thy^-$) were assayed for bioactivity with the CFC assay. RNA profiling (Thy^+ vs Thy^-) was then carried out to identify genes differentially expressed in stem cells. Results of the profiling are shown in Table 1. The data indicate that the upregulated genes encode proteins with diverse biochemical and cellular functions.

In addition, genes upregulated in $CD34^+Thy^+$ HSCs from two different sources, bone marrow and peripheral blood, were compared for overlapping sequences that are enriched in HSCs from both sources. A total of 30 genes were found to have been upregulated in HSCs from both sources. An exemplary list of these genes is shown in Table 2. Both HSC types contain transcription factors some of which are known proto-oncogenes (e.g., GATA3, HLF, Evi1, PMX1, MN1, ATF3).

Further, the results indicate that HSCs from peripheral blood, but not HSCs from bone marrow, are enriched in histones and inhibitory HLH transcription factors (ID1, ID2, and ID3). The data also suggest new cell surface markers for HSCs. Examples include 5T4, EphA3, TNFSF3, EVI2b, DLK1. Several potential neuropeptides are also upregulated, including Vasopression (AVP), Oxytocin (OXT), and Vasodilators.

Example 2. Inhition of HSC Differentiation By Overexpressing an HSC Differentiation-Inhibiting Polypeptide

The Example describes effects on HSC differentiation by constitutive expression of an HSC differentiation-inhibiting gene in CD34+Thy+ cells using retroviral vectors. First, effect of overexpressing ID3 was analyzed with colony-forming cell (CFC) assay. Other assays such as cobblestone area forming cell (CAFC) assay and NOD/SCID (nonobese diabetic mice with severe combined immunodeficiency disease) repopulating cell assay can also be used in these analyses. These assays can be performed as described as

described above and are well known in the art (e.g., Kusadasi et al., Leukemia 14: 1944-53, 2000; and Larochelle et al., Nature Medicine, 2: 1329-1337, 1996).

Fig. 1 illustrates the schematic structure of the retroviral vectors used in the study. Gene X in the figure denotes any of these HSC genes (e.g., ID3) to be examined. The vectors also express the green-fluorescence protein (GFP). When the GFP gene is transfected into or infected cells, the encoded GFP shines green under ultraviolet light and thus enables the detection of the transfected or infected cell in a simple manner.

A vector harboring the HSC gene (e.g., ID3 or GATA3) was transfected into the $CD34^+$ cells. Cells expressing the gene were sorted and assayed with the CFC assay. As shown in Fig. 2, ID3 over-expression increased the number of colony forming cells (e.g., primitive BFU-E colonies). This suggests enhanced HSC activity, indicating that differentiation of the stem cells has been slowed down.

The HSC differentiation-inhibiting genes were also examined for their effects on HSC growth in liquid culture. The effect of GATA3 over-expression on human HSC differentiation was examined in liquid culture. Here, stem cells were transfected with the same vectors described above (which harbor the ID1 gene, GATA3 gene, or no HSC gene), and grown in liquid culture. $CD34^+$ and GFP^+ cells were sorted. Expression of CD34 was monitored during the culture. Cells without transfection were used in a control analysis. The results indicate that, as compared to the control, ID1 had no effect on differentiation of the $CD34^+$ cells. However, expression of GATA3 significantly slowed the differentiation process as indicated by the rate of reduction of $CD4^+$ cells.

Example 3. Novel Molecular Markers Expressed in mouse HSCs

This Example describes use of RNA expression profiling to characterize purified mouse HSCs. Mouse HSCs were purified using a combination of antibodies to cell-surface markers. The following three cell populations were purified from murine bone marrow as described in Zhao et al., Blood 96: 3016-22, 2000; and Zhong et al., Blood 100: 3521-6, 2002.

Cell type	Immunophenotype	HSC activity
LT-HSC	Lin^-,c-kit^+,$Sca\text{-}1^+$,$CD38^+$, $CD34^-$	1X

Facilitator Cells	Lin^-,c-kit^+,Sca-1^+,$CD38^-$, $CD34^+$	0.1X
Progenitor Cells	Lin^-,c-kit^+,Sca-1^+,$CD38^+$, $CD34^+$	0.1X

Cells were purified from normal BL6 mice using flow cytometry. Three different preparations of sorted cells for each population were prepared and combined prior to the isolation of total RNA. The RNA was quantified using the Ribogreen fluorescence-based solution assay (e.g., as described in Jones et al., Anal Biochem 265: 368-74, 1998). 10ng of each pooled RNA preparation was labeled in duplicates using the triple labeling procedure (as described, e.g., in Hrabovszky et al., J. Histochem. Cytochem. 43: 363-370, 1995) and hybridized to affymetrix U74A gene chips according to the manufacturer's instructions. Intensity values were obtained for each gene and sample using GeneChip software. These Average difference (AD) values were exported to a spreadsheet program and analyzed by first filtering for genes which are expressed above a threshold criteria (50 in at least two samples), and whose average for each population was expressed >2X or < 2X between any two cell populations and where ANOVA analysis showed a significant difference ($P<0.01$) between any two populations.

Examples of genes upregulated in HSCs are shown in Table 3. The genes were analyzed for patterns using Genespring software and arranged by functional gene classification using GO ontogeny. Accession numbers or identification numbers from other public databases of these genes, as well as levels of up-regulation of these genes in HSCs as compared to non-HSCs, are also shown in the table.

Example 4. Characterization of Genes Differentially Expressed in mouse HSCs

To correlate stem cell activity of the three subsets with gene expression, a hypothetical stem cell activity pattern corresponding to the in vivo repopulating activity of the three subsets was generated and used for comparison of the normalized expression levels of each differentially expressed gene identified above. Principle Component Analysis (PCA) on the stem cell expression data was performed to identify gene expression patterns. This is an unsupervised computational method used to identify major patterns in diverse data types including gene expression data (Alter et al., Proc Natl Acad Sci USA 97:10101-10106, 2000; and Holter et al., Proc Natl Acad Sci USA 97:8409-8414, 2000). The correlation analysis of the gene expression patterns of the differentially expressed genes with stem cell

activity identified genes with highly significant (Pearson R >0.95) correlations. These genes are shown in Table 4. In addition to genes upregulated in HSCs, the analysis also identified genes whose expression negatively correlated with LTR HSCs (i.e., down-regulated expression). Examples of these genes are shown in Table 5.

Some of the differentially expressed genes were further analyzed and classified according to their biological functions. The results are shown in Table 6. As shown in Tables 3, 4, and 6, the upregulated genes in mouse HSCs also encode proteins of diverse biological properties, similar to genes upregulated in the human HSCs. For example, a number of transmembrane proteins were enriched in the mouse HSCs, as exemplified in Table 7. These molecules can be useful as novel surface markers for isolating HSCs. Some of transcription factors that are upregulated in the mouse HSCs are shown in Table 8. Their upregulated expression levels in the $CD34^{-}CD38^{+}$ HSCs relative to that in the facilitator cells ($CD38^{-}CD34^{+}$) and progenitor cells ($CD34^{+}CD38^{+}$) are shown in Figure 3.

The expression of several known transcription regulation factors was found to correlate positively with LTR HSC activity. These include Cited2, GATA3, Hdac3, Irf6, Jun B, Nmyc1, Rnps1, Xbp1, and Zfp292. Little is known regarding the role of these specific transcription factors in the control of HSC biology. These essential transcription factors could play an important role in regulating HSC development and differentiation.

To determine if any of the differentially expressed transcription factors are themselves regulating transcription in LTR HSCs, we performed a search of putative upstream regulatory regions (10 kb upstream of start codons) of the interrogated genes for binding sites of the nine transcription factors. Statistical analysis of these results revealed that only the binding sites of GATA were significantly enriched ($P<0.05$) within the differentially expressed genes. Interestingly, this list contains a large fraction (20 of 52) of the genes whose expression positively correlated with HSC activity, suggesting the possibility that Gata may play an important role in the control of LTR HSC biology. A small number of gene (3 of 20) whose expression is negatively correlated with HSC activity also contained Gata binding sites, suggesting the possibility that low levels of Gata expressed in STR HSC may influence gene expression at later stages.

To confirm the data from expression profiling, we performed semi-quantitative RT-PCR on total RNA extracted from the three BM subsets for three of the LTR HSC genes identified. These included the transcription factors Gata 3, Jun B, and the

thrombopoietin receptor c-Mpl. The results demonstrated that all three mRNAs are expressed at significantly higher levels in $CD38^{+}CD34^{-}$ cells compared to the other two subsets.

Table 3. Genes Upregulated in Mouse HSCs

Symbol	Description	RefSeq	Swiss Prot Keywords	HSC/non-HSC
AU044919	expressed sequence AU044919	AU044919	Glycoprotein Immunoglobulin C region Immunoglobulin domain	79.7
Klf2	Kruppel-like factor 2 (lung)	NM_008452	Activator DNA-binding Metal-binding Nuclear protein Repeat Transcription regulation Zinc-finger	44.9
Car1	carbonic anhydrase 1	NM_009799	Lyase Zinc	36.8
Mm.220154	Mus musculus anti-HIV-1 reverse transcriptase single-chain variable fragment mRNA, complete cds	NA	None	30.1
2010309G21Rik	RIKEN cDNA 2010309G21 gene	none	Immunoglobulin C region Immunoglobulin domain	28.8
NA	M80423:Mus castaneus IgK chain gene, C-region, 3 end /cds=(0,322) /gb=M80423 /gi=196865 /ug=Mm.46804 /len=323 mRNA	M80423	None	20.9
Fragilis	Fragilis	NM_025378	None	17.1
Smoc1	SPARC related modular calcium binding 1	NM_022316	None	15.8
5830413E08Rik	RIKEN cDNA 5830413E08 gene	NM_029083	None	14.9
5830431A10Rik	RIKEN cDNA 5830431A10 gene	none	None	14.4
AI325941	expressed sequence AI325941	AI325941	None	14.2
Cdkn1c	cyclin-dependent kinase inhibitor 1C (P57)	NM_009876	Alternative splicing Cell cycle	14.1
Lisch7	liver-specific bHLH-Zip transcription factor	none	None	13.9
AW108012	expressed sequence AW108012	AW108012	None	13.8
Akr1c13	aldo-keto reductase family 1, member C13	NM_013778	None	13.3
0910001L24Rik	RIKEN cDNA 0910001L24 gene	NM_022419	None	12.7
AI842353	expressed sequence AI842353	AI842353	None	11.7
Tgm2	transglutaminase 2, C polypeptide	NM_009373	Acyltransferase Calcium-binding Transferase	11.4
Nckap1	NCK-associated protein 1	none	Transmembrane	11.3
Serpina3g	serine (or cysteine) proteinase inhibitor, clade A, member 3G	none	None	11.3
1700008C22Rik	RIKEN cDNA 1700008C22 gene	none	None	10.4
Nmyc1	neuroblastoma myc-related oncogene 1	NM_008709	DNA-binding Nuclear protein Phosphorylation Proto-oncogene	10.4
Zfhx1a	zinc finger homeobox 1a	NM_011546	Activator DNA-binding Homeobox Metal-binding Nuclear protein Repeat Repressor Transcription regulation Zinc-finger	10.4
H2-Eb1	histocompatibility 2, class II antigen E beta	NM_010382	Glycoprotein MHC II Signal Transmembrane	10.0
AU044919	expressed sequence AU044919	AU044919	Glycoprotein Immunoglobulin C region Immunoglobulin domain	9.9

Gbp2	guanylate nucleotide binding protein 2	NM_010260	None	9.5
Gabbr1	gamma-aminobutyric acid (GABA-B) receptor, 1	NM_019439	Alternative splicing Coiled coil G-protein coupled receptor Glycoprotein Postsynaptic membrane Repeat Signal Transmembrane	9.5
D8Ertd69e	DNA segment, Chr 8, ERATO Doi 69, expressed	none	None	9.2
Gata3	GATA binding protein 3	NM_008091	Activator DNA-binding Nuclear protein T-cell Transcription regulation Zinc-finger	9.1
C130052I12Rik	RIKEN cDNA C130052I12 gene	NM_146047	None	8.7
0610025I19Rik	RIKEN cDNA 0610025I19 gene	NM_029555	None	8.6
Tcf15	transcription factor 15	NM_009328	None	8.6
H2-Aa	histocompatibility 2, class II antigen A, alpha	NM_010378	3D-structure Glycoprotein MHC II Signal Transmembrane	8.5
Tal1	T-cell acute lymphocytic leukemia 1	NM_011527	Chromosomal translocation Differentiation DNA-binding Phosphorylation Proto-oncogene Transcription regulation	8.3
Myoz1	myozenin 1	NM_021508	None	7.9
4930421J07Rik	RIKEN cDNA 4930421J07 gene	none	None	7.4
Igh-6	immunoglobulin heavy chain 6 (heavy chain of IgM)	none	Alternative splicing Glycoprotein Immunoglobulin C region Immunoglobulin domain Transmembrane	7.3
Hoxb5	Homeo box B5	NM_008268	Developmental protein DNA-binding Homeobox Nuclear protein Transcription regulation	7.3
Col9a1	procollagen, type IX, alpha 1	NM_007740	Alternative splicing Cartilage Collagen Connective tissue Extracellular matrix Glycoprotein Hydroxylation Repeat Signal	7.2
Meis1	myeloid ecotropic viral integration site 1	NM_010789	None	7.1
Ela1	elastase 1, pancreatic	none	None	7.0
Hiat1	hippocampus abundant gene transcript 1	NM_008246	None	7.0
Fah	fumarylacetoacetate hydrolase	NM_010176	Hydrolase Phenylalanine catabolism Tyrosine catabolism	6.9
Cypf13	cytochrome P450 CYP4F13	NM_130882	None	6.7
NA	:Mus musculus transcription factor PBX3b (PBX3b) mRNA, complete cds /cds=(118,1173) /gb=AF020200 /gi=2432016 /ug=Mm.7331 /len=2467 mRNA	AF020200	None	6.5
Igj	immunoglobulin joining chain	NM_152839	Glycoprotein Signal	6.3
NA	:AV336991 Mus musculus cDNA, 3 end /clone=6332407A01 /clone_end=3 /gb=AV336991 /gi=6377043 /ug=Mm.99212 /len=201 /NOTE=replacement for probe set(s) 100264_f_at on MG-	AV336991	None	6.2

	U74A mRNA			
Ctla2b	cytotoxic T lymphocyte-associated protein 2 beta	none	Repeat Signal T-cell	6.1
Serpinb6	serine (or cysteine) proteinase inhibitor, clade B, member 6	NM_009254	Serine protease inhibitor Serpin	5.8
Mm.29940	ESTs	NA	None	5.8
AU043625	expressed sequence AU043625	NM_133910	None	5.8
Col4a1	procollagen, type IV, alpha 1	none	Basement membrane Collagen Connective tissue Extracellular matrix Glycoprotein Hydroxylation Repeat Signal	5.6
Igh-4	immunoglobulin heavy chain 4 (serum IgG1)	none	Alternative splicing Glycoprotein Immunoglobulin C region Immunoglobulin domain	5.5
Siat6	sialyltransferase 6 (N-acetyllacosaminide alpha 2,3-sialyltransferase)	NM_009176	Glycoprotein Glycosyltransferase Golgi stack Signal-anchor Transferase Transmembrane	5.4
Igk-C	immunoglobulin kappa chain, constant region	none	None	5.4
Sdpr	Serum deprivation response	NM_138741	None	5.4
Dusp1	dual specificity phosphatase 1	NM_013642	Cell cycle Hydrolase	5.3
Cited2	Cbp/p300-interacting transactivator, with Glu/Asp-rich carboxy-terminal domain, 2	NM_010828	Alternative splicing Nuclear protein	5.2
Epor	erythropoietin receptor	NM_010149	Glycoprotein Receptor Signal Transmembrane	5.1
Mm.200980	Mus musculus, Similar to translocation protein 1, clone IMAGE:5347105, mRNA, partial cds	NA	None	5.0
Atf2	activating transcription factor 2	none	Activator Alternative splicing DNA-binding Metal-binding Nuclear protein Phosphorylation Transcription regulation Zinc-finger	5.0
Ccne1	cyclin E1	NM_007633	Cell cycle Cell division Cyclin Nuclear protein Phosphorylation	5.0
Mllt3	myeloid/lymphoid or mixed lineage-leukemia translocation to 3 homolog (Drosophila)	NM_027326	None	4.9
D5Ertd40e	DNA segment, Chr 5, ERATO Doi 40, expressed	none	None	4.9
Zfp216	zinc finger protein 216	NM_009551	None	4.8
Syp	synaptophysin	NM_009305	Calcium-binding Glycoprotein Nerve Phosphorylation Repeat Synapse Synaptosome Transmembrane	4.8
Nedd4	neural precursor cell expressed, developmentally down-regulted gene 4	NM_010890	Ligase Repeat Ubiquitin conjugation	4.7
Pbx1	pre B-cell leukemia transcription factor 1	NM_008783	None	4.7
6330407G11Rik	RIKEN cDNA 6330407G11 gene	NM_023423	None	4.6
Ash1	absent, small, or homeotic discs 1 (Drosophila)	NM_138679	None	4.5
Lmp	lymphoid-restricted membrane protein	NM_008511	None	4.5
Casp8ap2	caspase 8 associated protein 2	NM_011997	None	4.5

Mm.30163	Mus musculus, clone IMAGE:4952607, mRNA	NA	None	4.5
Ctsl	cathepsin L	NM_009984	Glycoprotein Hydrolase Lysosome Signal Thiol protease Zymogen	4.5
Sfpq	splicing factor proline/glutamine rich (polypyrimidine tract binding protein associated)	NM_023603	None	4.4
2010004A03Rik	RIKEN cDNA 2010004A03 gene	none	None	4.3
Car2	carbonic anhydrase 2	NM_009801	Lyase Zinc	4.2
Mm.22896	ESTs	NA	None	4.1
AI573938	expressed sequence AI573938	none	None	3.9
Vasp	vasodilator-stimulated phosphoprotein	none	Actin-binding Phosphorylation	3.9
AA408451	expressed sequence AA408451	AA408451	None	3.7
Pftk1	PFTAIRE protein kinase 1	NM_011074	None	3.6
Tieg	TGFB inducible early growth response	NM_013692	DNA-binding Metal-binding Nuclear protein Repeat Repressor Transcription regulation Zinc-finger	3.6
Igk-V28	immunoglobulin kappa chain variable 28 (V28)	none	Immunoglobulin C region Immunoglobulin domain	3.6
Mm.1806	Mus musculus, Similar to KIAA1404 protein, clone IMAGE:5252426, mRNA, partial cds	NA	None	3.5
Mm.25115	ESTs	NA	None	3.5
Ccm4l	CCR4 carbon catabolite repression 4-like (S. cerevisiae)	none	Biological rhythms	3.5
Cpo	coproporphyrinogen oxidase	NM_007757	Heme biosynthesis Iron Mitochondrion Oxidoreductase Porphyrin biosynthesis Transit peptide	3.5
Nupr1	nuclear protein 1	NM_019738	None	3.5
Mm.5510	similar to gene overexpressed in astrocytoma [Homo sapiens]	NA	None	3.4
Rab33b	RAB33B, member of RAS oncogene family	NM_016858	Golgi stack GTP-binding Lipoprotein Prenylation Protein transport	3.4
9430065L19Rik	RIKEN cDNA 9430065L19 gene	NM_146083	None	3.4
Pgr	progesterone receptor	NM_008829	DNA-binding Nuclear protein Receptor Steroid-binding Transcription regulation Zinc-finger	3.4
LOC218490	similar to Transcription factor BTF3 (RNA polymerase B transcription factor 3)	NM_145455	Alternative splicing Nuclear protein Transcription regulation	3.4
4930434H03Rik	RIKEN cDNA 4930434H03 gene	none	None	3.3
Actn3	Actinin alpha 3	NM_013456	Actin-binding Multigene family Repeat	3.3
Mm.202311	Mus musculus, clone IMAGE:1379624, mRNA, partial cds	NA	GTP-binding Lipoprotein Membrane Multigene family Palmitate Transducer	3.3
Gtpi	interferon-g induced GTPase	NM_019440	None	3.3
Nat2	N-acetyltransferase 2 (arylamine N-acetyltransferase)	NM_010874	Acyltransferase Multigene family Polymorphism Transferase	3.3
Eya2	eyes absent 2 homolog (Drosophila)	none	Alternative splicing Developmental protein Multigene family	3.3
1110037N09Rik	RIKEN cDNA 1110037N09 gene	none	None	3.2
5033414D02Rik	RIKEN cDNA 5033414D02 gene	NM_026362	None	3.1
Mm.26147	ESTs	NA	None	3.1

Il4	interleukin 4	NM_021283	B-cell activation Cytokine Glycoprotein Growth factor Signal	3.1
Ubap1	ubiquitin-associated protein 1	NM_023305	None	3.1
Acox1	acyl-Coenzyme A oxidase 1, palmitoyl	NM_015729	FAD Fatty acid metabolism Flavoprotein Oxidoreductase Peroxisome	2.9
Ccl5	chemokine (C-C motif) ligand 5	NM_013653	Chemotaxis Cytokine Inflammatory response Signal T-cell	2.9
AW457192	expressed sequence AW457192	NM_134084	Cyclosporin Isomerase Mitochondrion Multigene family Rotamase Transit peptide	2.9
2610016K11Rik	RIKEN cDNA 2610016K11 gene	none	None	2.8
Fzd4	frizzled homolog 4 (Drosophila)	NM_008055	Developmental protein G-protein coupled receptor Glycoprotein Multigene family Signal Transmembrane	2.8
Pla2g4a	phospholipase A2, group IVA (cytosolic, calcium-dependent)	NM_008869	Calcium Hydrolase Lipid degradation Phosphorylation	2.8
Scin	scinderin	NM_009132	None	2.7
NA	AV239653 Mus musculus cDNA, 3 end /clone=4732435F04 /clone_end=3 /gb=AV239653 /gi=6192160 /ug=Mm.88313 /len=214 /NOTE=replacement for probe set(s) 96411_f_at on MG-U74A mRNA	AV239653	None	2.7
Tcf12	transcription factor 12	NM_011544	Alternative splicing Developmental protein DNA-binding Nuclear protein Transcription regulation	2.7
Madh7	MAD homolog 7 (Drosophila)	NM_008543	Alternative splicing Multigene family Transcription regulation	2.7
Gem	GTP binding protein (gene overexpressed in skeletal muscle)	NM_010276	GTP-binding Membrane Phosphorylation	2.7
Tpm1	tropomyosin 1, alpha	NM_024427	3D-structure Acetylation Alternative splicing Coiled coil Multigene family Muscle protein Phosphorylation Repeat	2.7
Map17	membrane-associated protein 17	NM_026018	None	2.7
Dcx	doublecortin	NM_010025	Neurogenesis Neurone Phosphorylation Repeat	2.7
Igk-V28	immunoglobulin kappa chain variable 28 (V28)	none	Immunoglobulin C region Immunoglobulin domain	2.6
Rnf11	ring finger protein 11	NM_013876	None	2.6
Nfix	nuclear factor I/X	NM_010906	None	2.6
Lin7c	lin 7 homolog c (C. elegans)	NM_011699	None	2.5
Cln3	ceroid lipofuscinosis, neuronal 3, juvenile (Batten, Spielmeyer-Vogt disease)	NM_009907	Glycoprotein Lysosome Transmembrane	2.5
Hhex	hematopoietically expressed homeobox	NM_008245	Developmental protein DNA-binding Homeobox Nuclear protein	2.5
Gab1	growth factor receptor bound protein 2-associated protein 1	NM_021356	None	2.5
None	none	none	None	2.5
Kcnj3	potassium inwardly-rectifying	NM_008426	Ion transport Ionic channel	2.5

	channel, subfamily J, member 3		Potassium transport Transmembrane Voltage-gated channel	
Cradd	CASP2 and RIPK1 domain containing adaptor with death domain	NM_009950	Apoptosis	2.5
Mm.29914	ESTs	NA	None	2.4
Fos	FBJ osteosarcoma oncogene	NM_010234	DNA-binding Nuclear protein Phosphorylation Proto-oncogene	2.4
Mm.24247	ESTs	NA	None	2.4
4930472G13Rik	RIKEN cDNA 4930472G13 gene	NM_029447	None	2.4
Ormdl3	ORM1-like 3 (S. cerevisiae)	NM_025661	None	2.4
Umpk	uridine monophosphate kinase	none	Kinase Transferase	2.4
Creg	cellular repressor of E1A-stimulated genes	NM_011804	None	2.4
Utrn	utrophin	none	None	2.3
Mm.27769	ESTs, Weakly similar to RIKEN cDNA 0610011E17 [Mus musculus] [M.musculus]	NA	None	2.3
Igtp	interferon gamma induced GTPase	NM_018738	None	2.3
Arg2	arginase type II	NM_009705	Arginine metabolism Hydrolase Manganese Mitochondrion Transit peptide Urea cycle	2.3
Pklr	pyruvate kinase liver and red blood cell	NM_013631	Alternative splicing Glycolysis Kinase Magnesium Multigene family Phosphorylation Transferase	2.2
1810010A06Rik	RIKEN cDNA 1810010A06 gene	NM_026921	None	2.2
Mm.532	ESTs, Weakly similar to lysophospholipase 1; phospholipase 1a; lysophopholipase 1 [Mus musculus] [M.musculus]	NA	None	2.2
Vamp5	vesicle-associated membrane protein 5	NM_016872	Multigene family Myogenesis Signal-anchor Transmembrane	2.2
0710001O03Rik	RIKEN cDNA 0710001O03 gene	NM_146094	None	2.2
2610003J05Rik	RIKEN cDNA 2610003J05 gene	none	None	2.2
Tdel1	tumor differentially expressed 1, like	NM_019760	None	2.2
Serpinf1	serine (or cysteine) proteinase inhibitor, clade F), member 1	NM_011340	Glycoprotein Serpin Signal	2.1
Scotin	scotin gene	NM_025858	None	2.1
G3bp2	Ras-GTPase-activating protein (GAP<120>) SH3-domain binding protein 2	NM_011816	None	2.1
1190002H23Rik	RIKEN cDNA 1190002H23 gene	NM_025427	None	2.1
Nsccn1	non-selective cation channel 1	NM_010940	None	2.1
Tgoln2	trans-golgi network protein 2	NM_009444	None	2.1
Ywhae	tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, epsilon polypeptide	NM_009536	None	2.1
4631408O11Rik	RIKEN cDNA 4631408O11 gene	none	None	2.1
Pou2af1	POU domain, class 2, associating factor 1	NM_011136	Nuclear protein Transcription regulation	2.1
Mm.220953	Mus musculus, clone IMAGE:4206769, mRNA	NA	None	2.1
Casp6	caspase 6	NM_009811	Apoptosis Hydrolase Thiol protease Zymogen	2.0
None	none	none	Glycoprotein Immunoglobulin C region Immunoglobulin domain	2.0
Nr4a1	nuclear receptor subfamily 4, group A, member 1	NM_010444	DNA-binding Nuclear protein Phosphorylation Receptor Transcription	2.0

			regulation Zinc-finger	
1700023O11Rik	RIKEN cDNA 1700023O11 gene	NM_029339	None	2.0
Brca2	breast cancer 2	NM_009765	Polymorphism Repeat	2.0
H2-T22	histocompatibility 2, T region locus 22	NM_010397	None	2.0

Table 4 Genes With Upregulated Expression and Correlated Stem Cell Activity

Symbol or Acc. No.	Gene Description or similarity to known proteins	Correlation to stem cell	Unigene No.
Rnps1	ribonucleic acid binding protein S1	1.000	Mm.1951
Junb	Jun-B oncogene	1.000	Mm.1167
Hdac3	histone deacetylase 3	1.000	Mm.20521
Irf6	interferon regulatory factor 6	1.000	Mm.4179
Gata3	GATA binding protein 3	0.997	Mm.606
Xbp1	X-box binding protein 1	0.993	Mm.22718
Cited2	Cbp/p300-interacting transactivator, with Glu/Asp-rich carboxy-terminal domain, 2	0.992	Mm.9524
Nmyc1	neuroblastoma myc-related oncogene 1	0.986	Mm.16469
Zfp292	zinc finger protein 292	0.975	Mm.38193
Bdkrb1	bradykinin receptor, beta 1	1.000	Mm.57076
Map17	membrane-associated protein 17	0.995	Mm.30181
Ormdl3	ORM1-like 3 (S. cerevisiae)	0.990	Mm.180546
Fzd4	frizzled homolog 4 (Drosophila)	0.988	Mm.68712
Lgi4	leucine-rich repeat LGI family, member 4	0.961	Mm.1662
Bdkrb1*	bradykinin receptor, beta 1	1.000	Mm.57076
Socs2	suppressor of cytokine signaling 2	0.996	Mm.4132
Fzd4*	frizzled homolog 4 (Drosophila)	0.988	Mm.68712
Kit*	kit oncogene	0.961	Mm.4394
Inpp5d	inositol polyphosphate-5-phosphatase D	0.958	Mm.15105
Fbxo9	f-box only protein 9	1.000	Mm.28584
Nedd4	neural precursor cell expressed, developmentally down-regulted gene 4	0.993	Mm.16553
Rnf11	ring finger protein 11	0.992	Mm.25228
Ian1	immune associated nucleotide 1	0.999	Mm.28395
Iigp	interferon-inducible GTPase	0.997	Mm.29008
Ifi47	interferon gamma inducible protein	0.984	Mm.24769
Tgtp	T-cell specific GTPase	0.994	Mm.15793
Igtp	interferon gamma induced GTPase	0.993	Mm.858
Gtpi	interferon-g induced GTPase	0.989	Mm.33902
Serpinb6a	serine (or cysteine) proteinase inhibitor, clade B, member 6a	0.996	Mm.2623
Serpina3g	serine (or cysteine) proteinase inhibitor, clade A, member 3G	0.987	Mm.15085
Camk2b	calcium/calmodulin-dependent protein kinase II, beta	0.999	Mm.4857
Gab1	growth factor receptor bound	0.997	Mm.24573

	protein 2-associated protein 1		
Gabarapl1	gamma-aminobutyric acid (GABA(A)) receptor-associated protein-like 1	0.997	Mm.14638
Mtmr13	myotubularin related protein 13	0.996	Mm.200250
Mt2	metallothionein 2	0.999	Mm.147226
Car2	carbonic anhydrase 2	0.995	Mm.1186
Cdkn1c	cyclin-dependent kinase inhibitor 1C (P57)	0.986	Mm.168789
Lcn7	lipocalin 7	0.999	Mm.15801
A430017F18	No similar gene	1.000	Mm.44883
AU044919	No significant similar gene	1.000	Mm.14438
2310075M17Rik	Similar to S3543 GTP-binding protein (90%)	0.999	Mm.196592
Ell2	Eleven-nineteen lysine-rich leukemia gene 2	0.998	Mm.21288
LOC207685	Hypothetical protein	0.998	Mm.38214
2310061I04Rik	No similar gene	0.998	Mm.5624
5830431A10Rik	Contain Cor1/Xlr/Xmr conserved region	0.997	Mm.1148
2700007P21Rik	Unknown protein	0.997	Mm.3587
B930086G17	No similar gene	0.992	Mm.24738
2410166I05Rik	Hypothetical protein	0.989	Mm.30153
D10Ertd749e	Similar to ZW10 interacting protein-1	0.986	Mm.38994
2210023F24Rik	Contain B-box Zn-finger and SPRY domain	0.983	Mm.5510
Riken 4237666	No significant similar gene	0.978	Mm.276231
6230421P05Rik	No similar gene	0.978	Mm.26147
4631408O11Rik*	No significant similar gene	0.964	Mm.2935
1110054N06Rik*	Unknow protein with Ankyrin repeat	0.960	Mm.15351

Table 5 Genes down-regulated in CD38+CD34- Cells

Symbol or Acc. No.	Description	Correlation to SC activity	Unigene No.
Satb1	Special AT-rich sequence binding protein 1	0.955	Mm.4381
Ptpro	Protein tyrosine phosphatase, receptor type, O	0.999	Mm.4715
Sell	Selectin, lymphocyte	0.988	Mm.1461
Ccl9	Chemokine (C-C motif) ligand 9	0.988	Mm.2271
Cnn3	Calponin 3, acidic	0.988	Mm.22171
Lgals3	Lectin, galactose binding, soluble 3	0.971	Mm.2970
Mki67	Antigen identified by monoclonal antibody Ki 67	0.998	Mm.4078
Bin1	Bridging integrator 1	0.977	Mm.4383
Sult4a1	Sulfotransferase family 4A, member 1	1.000	Mm.20451
Hdc	Histidine decarboxylase	0.996	Mm.18603
AI132321	Contain phospholipase D. active site motif	-1.000	Mm.203915
2610036L13Rik	No similar gene	-1.000	Mm.23526
BC018347	Similar to translation-initiation factor IF-2	-1.000	Mm.154309
X90778	Similar to Histone H2B	-1.000	Mm.21579
AW060549	Similar to Retrovirus-related POL polyprotein	-0.999	Mm.29177

X67863	Similar to Octapeptide-repeat protein T2	-0.995	Mm.35868
X15378	Similar to Myeloperoxidase and Eosinophil peroxidase precursor	-0.975	Mm.4668
Plac8	Uncharacterized Cys-rich domain containing protein	-0.960	Mm.34609
D13Ertd275e	Hypothetical protein	-0.952	Mm.21231

Table 6. Cassification and Characterization of Genes Upregulated in Mouse HSCs

Class	Name	Sequence Description	Sequence Code	Unigene Code	Protein ID
Apoptosis	Birc5	baculoviral IAP repeat-containing 5	101521	Mm.8552	O70201
Cell cycle	Spin	spindlin	99563	Mm.42193	
Chromosomal	Btg1	M.musculus btg1 mRNA.	93104		P31607
Chromosomal	Calm2	Mus musculus calmodulin synthesis (CaM) cDNA, complete cds.	93293		P02593
Enzyme	Ctsl	cathepsin L	101963	Mm.930	P06797
Enzyme	Gdi1	guanosine diphosphate (GDP) dissociation inhibitor 1	97313	Mm.205830	P50396
Enzyme	Hadh2	hydroxysteroid (17-beta) dehydrogenase 10	101045	Mm.6994	O08756
Enzyme	Mt2	Mouse metallothionein II (MT-II) gene.	101561		P02798
Enzyme	Pnp	purine-nucleoside phosphorylase	93290	Mm.17932	P23492
Enzyme	Vdu1	Vhlh-interacting deubiquitinating enzyme 1	160710	Mm.24383	
Kinase	Csnk1e	casein kinase 1, epsilon	97925	Mm.30199	Q9QUI3
Kinase	Nme3	expressed in non-metastatic cells 3	94981 i	Mm.27278	
Lectin	Lgals9	lectin, galactose binding, soluble 9	103335	Mm.18087	O08573
Metabolism	Aldh1a1	aldehyde dehydrogenase family 1, subfamily A1	100068	Mm.4514	P24549
Metabolism	Aldh1a7	aldehyde dehydrogenase family 1, subfamily A7	94778	Mm.14609	O35945
Metabolism	Cpo	coproporphyrinogen oxidase	98505 i	Mm.35820	P36552
Metabolism	Cpo	coproporphyrinogen oxidase	98506 r	Mm.35820	P36552
Metabolism	Ech1	enoyl coenzyme A hydratase 1, peroxisomal	93754	Mm.2112	O35459
Metabolism	Mtcp1	M.musculus MTCP-1 gene.	103043		Q61908
Nuclear	Rbmx	RNA binding motif protein, X chromosome	97848	Mm.28275	Q9R0Y0
Nuclear	Snrpa	small nuclear ribonucleoprotein polypeptide A	100101	Mm.4633	Q62189
Secreted	Iap	intracisternal A particles	97181 f	Mm.212712	P03975
Secreted	Tff2	Mus musculus spasmolytic polypeptide (mSP) gene, complete cds.	93302		Q03404
Signaling	Gnb4	guanine nucleotide binding protein, beta 4	93949	Mm.9336	P29387
Signaling	Tsc2	tuberous sclerosis 2	97953 g	Mm.30435	Q61037
Structural	Fscn1	fascin homolog 1, actin bundling protein (Strongylocentrotus) purpuratus)	92838	Mm.13194	Q61553
Transcription	Irf1	Interferon regulatory factor 1	102401	Mm.1246	P15314
Transcription	Cited2	Cbp/p300-interacting transactivator, with Glu/Asp-rich carboxy-terminal domain, 2	101973	Mm.9524	O35740
Transcription	Ncor1	nuclear receptor co-repressor 1	101536	Mm.88061	Q60974
Transcription	Sox6	SRY-box containing gene 6	92726	Mm.4656	P40645
Transcription	Hhex	Mus musculus Hex(Prh) gene, exon4 and complete cds.	98408	Mm.33896	Q9R1X2
Transcription	Trim30	tripartite motif protein 30	98030	Mm.3288	P15533
Transcription	Tieg	TGFB inducible early growth response	99602	Mm.4292	O89091
Transcription	Klf2	Kruppel-like factor 2 (lung)	96109	Mm.26938	Q60843
Transcription	Eif4a2	eukaryotic translation initiation factor 4A2	93089	Mm.16323	P10630
Transcription	H2a-615	Mus musculus histone H2a.2-615 (H2a-615), and histone H3.2-615 (H3-615) genes, complete cds.	93068_r		P20670
Transcription	Nfe2l2	Mus musculus p45 NF-E2 related factor 2 (NRF2) gene, exon 2 to exon 5 and	92562		Q60795

		complete cds.			
Transcription	Fli1	Friend leukemia integration 1	94698	Mm.119781	P26323
Transcription	Mcmd5	mini chromosome maintenance deficient 5 (S. cerevisiae)	100156	Mm.5048	P49718
Transcription	H3f3b	H3 histone, family 3B	100708	Mm.18516	P06351
Transcription	Rev3l	REV3-like, catalytic subunit of DNA polymerase zeta RAD54 like (S. cerevisiae)	103457	Mm.2167	Q61493
Transcription	Hoxb5	homeo box B5	103666	Mm.207	P09079
Transcription	Pbx1	pre B-cell leukemia transcription factor 1	94804	Mm.221246	P41778
Transcription	Zfp36l1	zinc finger protein 36, C3H type-like 1	93324	Mm.18571	P23950
Transcription	Myb	myeloblastosis oncogene	92644_s	Mm.1202	P06876
Transcription	Sp4	trans-acting transcription factor 4	92992_i	Mm.5073	
Transcription	Idb2	Mus musculus helix-loop-helix protein Id2 gene, 3' region.	93013		
Transmembrane	Hiat1	hippocampus abundant gene transcript 1	160447	Mm.3792	P70187
Transmembrane	Igh-4	mouse gene for the constant part of gamma-1 immunogloblin.	101870		P01869
Transmembrane	Ii	Ia-associated invariant chain	101054	Mm.7043	P04441
Transmembrane	H2-Aa	histocompatibility 2, class II antigen A, alpha	92866	Mm.175310	P23150
Transmembrane	Epor	Mouse gene for erythropoietin receptor.	103997		P14753
Transmembrane	Irs2	Mus musculus insulin receptor substrate-2 (Irs2) gene, partial cds.	92205		O88970
Transmembrane	H2-Eb1	histocompatibility 2, class II antigen E beta	94285	Mm.22564	Q61857
Transmembrane	Tnfrsf17	tumor necrosis factor receptor superfamily, member 17	94190	Mm.12935	O88472
Transmembrane	Adcy9	adenylate cyclase 9	92527	Mm.4294	P51830
Transmembrane	Edg1	endothelial differentiation sphingolipid G-protein-coupled receptor 1	161788_f	Mm.982	
Transmembrane	Fzd4	frizzled homolog 4 (Drosophila)	93459_s	Mm.68712	
Transport	Vps35	vacuolar protein sorting 35	92640	Mm.196201	Q9EQH3
Transport	Hbb-b2	Mouse gene for beta-1-globin.	103534		P02089
Transport	Kpnb1	karyopherin (importin) beta 1	93111	Mm.16710	P70168
Transport	Rab9	RAB9, member RAS oncogene family	95516	Mm.25306	Q9R0M6
Transport	Rac1	RAS-related C3 botulinum substrate 1	101555	Mm.889	P15154
Transport	Rab33b	Mus musculus DNA for Rab33B, exon 2 and complete cds.	103062		O35963
Zinc Finger	Zfp216	zinc finger protein 216	160321	Mm.2904	O88878
Zinc Finger	Rnf11	ring finger protein 11	160205_f	Mm.25228	Q9QYK7
Zinc Finger	Nbr1	next to the Brca1	101484	Mm.784	P97432
Zinc Finger	pol	Mus musculus clone MIA14 full-length intracisternal A-particle gag protein gene, complete cds; and pol pseudogene, complete sequence.	93907_f		P11365
Zinc Finger	Gfi1b	growth factor independent 1B	102260	Mm.10804	O70237
Zinc Finger	Car1	carbonic anhydrase 1	98098	Mm.3471	P13634
	Cul4a	cullin 4A	104288	Mm.22276	
	D7Wsu128e	DNA segment, Chr 7, Wayne State University 128, expressed	103861_s	Mm.21103	
	Rhced	Rhesus blood group CE and D	103340	Mm.195461	Q9QX04
	AU044919	expressed sequence AU044919	102823	Mm.14438	
	Igj	immunoglobulin joining chain	102372	Mm.1192	
	Lisch7	liver-specific bHLH-Zip transcription factor	162274_f	Mm.4067	
	Igh-VJ558	immunoglobulin heavy chain, (J558 family)	161486_f	Mm.157783	
	0910001L24 Rik	RIKEN cDNA 0910001L24 gene	161243_f	Mm.22637	
	Txnip	thioredoxin interacting protein	160547_s	Mm.77432	
	Dr1	down-regulator of transcription 1	160449	Mm.38184	
	4933429H19 Rik	Mus musculus, Similar to translocation protein 1, clone IMAGE:5347105, mRNA, partial cds	160136_r	Mm.200980	
	1500010B24 Rik	RIKEN cDNA 1500010B24 gene	160111	Mm.65264	
	IgM	Mus castaneus IgK chain gene, C-region, 3'	102156_f		

		end.			
	AA409749	expressed sequence AA409749	100742	Mm.3628	
	D2Ertd63e	DNA segment, Chr 2, ERATO Doi 63, expressed	95862	Mm.24965	
	Igk-V28	Mus musculus anti-HIV-1 reverse transcriptase single-chain variable fragment mRNA, complete cds	100322	Mm.220154	
	5830431A10 Rik	RIKEN cDNA 5830431A10 gene	94136	Mm.1148	
	Igl-V1	Mouse Ig active lambda-1-chain C-region gene, 3' end.	93638_s		
	Imap38	immunity-associated protein, 38 kDa	92489	Mm.197478	P70224
	92316_f	Mouse germline Ig lambda-2-chain C-region gene, 3' end.	92316_f		
	2700007P21 Rik	RIKEN cDNA 2700007P21 gene	92268	Mm.3587	
	104477	ESTs	104477	Mm.29940	
	0610012A05 Rik	RIKEN cDNA 0610012A05 gene	104206	Mm.27619	
	Atp6s1	Mus musculus, clone MGC:37615 IMAGE:4989784, mRNA, complete cds	103699_i	Mm.222723	
	Gbp3	guanylate nucleotide binding protein 3	103202	Mm.1909	
	immunoglobulin V region	Mouse mRNA for immunoglobulin gamma-3 V-D-J region and secreted constant region, complete cds.	102721		
	AI256744	Mus musculus, clone IMAGE:3500612, mRNA, partial cds	102233	Mm.1043	
	Ptdss1	phosphatidylserine synthase 1	101931	Mm.9440	O55024
	Gga1	golgi associated, gamma adaptin ear containing, ARF binding protein 1	98445	Mm.34525	
	4121402D02 Rik	RIKEN cDNA 4121402D02 gene	97935	Mm.30252	
	Iigp	interferon-inducible GTPase	96764	Mm.29008	Q9Z1M3
	2310022K15 Rik	RIKEN cDNA 2310022K15 gene	95622	Mm.28047	
	Vcl	vinculin	94963	Mm.12842	
	2610319K07 Rik	RIKEN cDNA 2610319K07 gene	104744	Mm.200479	
	Iga	Mouse Ig germline D-J-C region alpha gene and secreted tail; Mouse germ line gene for immunoglobulin alpha H constant part (coding for the last three exons)	100583		
	Prpf8	pre-mRNA processing factor 8	98574	Mm.3757	
	Scotin	scotin gene	95102	Mm.196533	
	1110035L05 Rik	RIKEN cDNA 1110035L05 gene	95052	Mm.29140	
	3110001A13 Rik	RIKEN cDNA 3110001A13 gene	96640	Mm.200627	
	Vps26	vacuolar protein sorting 26 (yeast)	96665	Mm.27373	
	mu-immunoglobulin	Mouse germ line gene fragment for mu-immunoglobulin C-terminus (secreted form).	93583_s		
	H19	M.musculus H19 mRNA.	93028		Q61638
	Car2	carbonic anhydrase 2	92642	Mm.1186	
	Rae1	RAE1 RNA export 1 homolog (S. pombe)	160466	Mm.4113	
	Map1lc3	microtubule-associated protein 1 light chain 3	160288	Mm.28357	
	1700008C22 Rik	RIKEN cDNA 1700008C22 gene	160123	Mm.177990	
	98254_f	un98f06.x1 NCI_CGAP_Mam6 Mus musculus cDNA clone IMAGE:2581955 3' similar to gb:M10062 Mouse IgE-binding factor mRNA, complete cds (MOUSE); mRNA sequence.	98254_f		
	Eef2	eukaryotic translation elongation factor 2	97559	Mm.27818	Q61509

	Igk-V28	immunoglobulin kappa chain variable 28 (V28)	99405	Mm.104747	
	9030022E12 Rik	RIKEN cDNA 9030022E12 gene	104198	Mm.27519	
	D18362	expressed sequence D18362	103206	Mm.205433	
	Heyl	Mus musculus 6 days neonate head cDNA, RIKEN full-length enriched library, clone:5430408K11:hairy/enhancer-of-split related with YRPW motif 1, full insert sequence	101913	Mm.222825	
	shrm	shroom	100024	Mm.46014	
	AW547365	expressed sequence AW547365	97425	Mm.30015	
	D8Ertd69e	DNA segment, Chr 8, ERATO Doi 69, expressed	94922_i	Mm.26609	
	Frap1	FK506 binding protein 12-rapamycin associated protein 1	104708	Mm.21158	
	4933434E20 Rik	RIKEN cDNA 4933434E20 gene	104038	Mm.21451	
	1810009A16 Rik	RIKEN cDNA 1810009A16 gene	104041	Mm.21458	
	Pex11a	peroxisomal biogenesis factor 11a	103660	Mm.20615	Q9Z211
	AU044919	expressed sequence AU044919	102824_g	Mm.14438	
	MGC29044	hypothetical protein MGC29044	102375	Mm.1196	
	Mkrn1	makorin, ring finger protein, 1	101070	Mm.7198	
	LOC207933	similar to Isopentenyl-diphosphate delta-isomerase (IPP isomerase) (Isopentenyl pyrophosphate isomerase)	96269	Mm.29847	
	Elp3	elongation protein 3 homolog (S. cerevisiae)	95717	Mm.29719	
	Add1	adducin 1 (alpha)	94535	Mm.29052	
	Pbef	pre-B-cell colony-enhancing factor	94461	Mm.28830	
	4930588A18 Rik	Mus musculus, clone IMAGE:4457493, mRNA	96717	Mm.233830	
	Dad1	Mus musculus Defender against Apoptotic Death (Dad1) gene, exon 3.	96008		
	2410015A15 Rik	RIKEN cDNA 2410015A15 gene	95433	Mm.24495	
	Xbp1	X-box binding protein 1	94821	Mm.22718	
	Net1	neuroepithelial cell transforming gene 1	94223	Mm.22261	Q9Z1L7
	Igk-V28	immunoglobulin kappa chain variable 28 (V28)	93086	Mm.104747	
	LOC218490	similar to Transcription factor BTF3 (RNA polymerase B transcription factor 3)	93057	Mm.1538	
	Lamc1	laminin, gamma 1	161706_f	Mm.1249	
	AI450287	expressed sequence AI450287	161596_f	Mm.222827	
	Sep15	15-kDa selenoprotein	160360	Mm.29812	
	LOC229906	similar to TRANSCRIPTION INITIATION FACTOR IIB (TFIIB) (RNA POLYMERASE II ALPHA INITIATION FACTOR)	160225	Mm.27213	
	2810043O03 Rik	RIKEN cDNA 2810043O03 gene	98756	Mm.45532	
	96532	ESTs, Highly similar to nucleolar protein GU2 [Mus musculus] [M.musculus]	96532	Mm.35019	
	Myt1l	myelin transcription factor 1-like	96495	Mm.2523	P97500
	2010004A03 Rik	RIKEN cDNA 2010004A03 gene	94802	Mm.35302	
	C79248	expressed sequence C79248	94689	Mm.153895	
	Mylk	myosin, light polypeptide kinase	93482	Mm.27680	
	D1Ertd147e	DNA segment, Chr 1, ERATO Doi 147, expressed	93191	Mm.5572	
	R75364	expressed sequence R75364	92397	Mm.89393	
	92245	ESTs, Highly similar to nucleolar protein GU2 [Mus musculus] [M.musculus]	92245	Mm.35019	

	Ctse	Mus musculus cathepsin E gene, exon 1, partial.	104696		
	AA420392	expressed sequence AA420392	104670	Mm.32357	
	Acyp2	acylphosphatase 2, muscle type	104258	Mm.28407	
	Lrba	LPS-responsive beige-like anchor	104264	Mm.28458	
	Dock2	dedicator of cyto-kinesis 2	103462	Mm.2173	
	Gabpa	GA repeat binding protein, alpha	103440	Mm.18974	
	Nrip1	nuclear receptor interacting protein 1	103288	Mm.20895	Q9Z2K2
	AI225904	expressed sequence AI225904	103200	Mm.1902	
	98438_f	Mouse Q4 class I MHC gene (exon 5).	98438_f		Q31220
	2010012D11 Rik	RIKEN cDNA 2010012D11 gene	96231	Mm.140243	
	AU019574	Mus musculus, Similar to hypothetical protein FLJ11110, clone MGC:11734 IMAGE:3968418, mRNA, complete cds	96172	Mm.28395	
	9130415E20 Rik	RIKEN cDNA 9130415E20 gene	95020	Mm.40620	
	95021	Mus musculus, clone IMAGE:4502890, mRNA	95021	Mm.27476	
	AW495846	expressed sequence AW495846	104549	Mm.23702	
	Gtpbp2	GTP binding protein 2	104144	Mm.22147	
	2310050N11 Rik	RIKEN cDNA 2310050N11 gene	104114	Mm.21954	
	Ormdl3	ORM1-like 3 (S. cerevisiae)	98065	Mm.180546	
	2610003J05 Rik	RIKEN cDNA 2610003J05 gene	97491	Mm.31051	
	Map17	membrane-associated protein 17	96935	Mm.30181	
	Gabarapl2	GABA(A) receptor-associated protein like 2	96840	Mm.30017	
	2310050K10 Rik	RIKEN cDNA 2310050K10 gene	95743	Mm.29769	
	AI182287	expressed sequence AI182287	94469	Mm.28848	
	Nudel	nuclear distribution gene E-like	98884_r	Mm.31979	
	Cpne1	copine I	97199	Mm.27660	
	Dnajb9	DnaJ (Hsp40) homolog, subfamily B, member 9	96680	Mm.27432	
	95488	Mus musculus, clone IMAGE:3597827, mRNA, partial cds	95488	Mm.25018	
	2700059C12 Rik	RIKEN cDNA 2700059C12 gene	93312	Mm.18485	
	Sdcbp	syndecan binding protein	93017	Mm.14744	O88601

Table 7. Tansmembrane Proteins Enriched in Mouse HSCs

Classification	Description
surface antigen	Histocompatibility 2, class II antigen E beta
receptor	Gamma-aminobutyric acid (GABA) B receptor, 1
oncogene	Myeloproliferative leukemia virus oncogene (TPOR)
surface antigen	Histocompatibility 2, class II antigen A alpha
	Cytotoxic T lymphocyte-associated protein 2 beta
receptor	Erythropoietin receptor
oncogene	Kit oncogene
	Coagulation factor II (thrombin) receptor
	Frizzled homolog 4 (Drosophila)
	Membrane-associated protein 17
surface glycoprotein	ESTs similar to C211_Human putative surface glycoprotein

Table 8. Transcription Factors Upregulated in Mouse HSCs

Symbol	Description	Fold change	Accession No.
Klf2	Kruppel-like factor 2 (lung)	44.9	NM_008452
Nmyc1	neuroblastoma myc-related oncogene 1	10.4	NM_008709
Zfx1ha	zinc finger homeobox 1a	10.4	NM_011546
Gata3	GATA-binding protein 3	9.0	NM_008091
Tcf15	transcription factor 15	8.6	NM_009328
Tal1	T-cell acute lymphocytic leukemia 1	8.3	NM_011527
Hoxb5	homeo box B5	7.2	NM_008268
Meis1	myeloid ecotropic viral integration site 1	7.1	NM_010789
Pbx3b	Mus musculus transcription factor PBX3b	6.5	AF020200
Cited2	Cbp/p300-interacting transactivator 2	5.2	NM_010828
Atf2	activating transcription factor 2	3.6	none
Pbx1	pre B-cell leukemia transcription factor 1	4.7	NM_008783
None	chromatin remodeling factor	4.5	Mm.24637
None	EST similar to PRE-MRNA SPLICING FACTOR SRP20	3.4	Mm.29915
Btf3	basic transcription factor 3	3.2	none
Tcf12	transcription factor 12	2.7	NM_011544
Madh7	MAD homolog 7 (Drosophila)	2.7	NM_008543
Hhex	hematopoietically expressed homeobox	2.5	NM_008245

Example 5. Hierarchical Clustering Analysis of Differential Expressed Genes

This Example describes study aimed at determining if genes differentially expressed with the HSC compartment are also expressed in other tissues. To perform this analysis we compared the gene expression levels of 210 differentially expressed HSC genes with a database composed of 45 normal tissue. Hierarchical clustering of these data was used to group both those tissues and genes with similar expression patterns. The three HSC cell subsets formed a distinct branch in this analysis, with LTR-enriched $38^{+}34^{-}$ cells forming a discrete branch compared to the STR cells ($38^{+}34^{+}$ and $38^{-}34^{+}$). This clustering pattern is consistent with the stem cell activity pattern within the three subsets. Importantly, the HSC samples do not cluster near the bone or bone marrow samples suggesting that the differentially expressed HSC genes are not bone marrow related. This analysis also showed that the majority of these genes were not ubiquitously expressed although most were expressed at comparable levels in at least one other tissue.

Three of the genes were found to have their peak expression within the HSC compartment. These were the scaffolding protein Gab1 (GRB2-asssociated binding protein 1) and the uncharacterized gene A430017F18 which displayed the highest level expression in the LTR enriched $CD38^{+}CD34^{-}$ cells, and the Pdgfrb gene (platelet derived growth factor receptor, beta polypeptide) which peaked within the $38^{+}34^{+}$ STR HSC subset. Although the majority of these genes are also expressed at comparable levels in other tissues it is important to note that in many cases the level of expression in HSC subsets was at or near the peak expression determined for these genes across the entire 45 tissue panel. The high relative expression within HSCs of this subset of genes indicates that they likely to play an important role in the biology of HSCs.

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It is understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application and scope of the appended claims. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods and materials are described.

All publications, GenBank sequences, patents and patent applications cited herein are hereby expressly incorporated by reference in their entirety and for all purposes as if each is individually so denoted.

WE CLAIM:

1. A method for inhibiting differentiation of mammalian stem cells, comprising (a) providing a population of stem cells, (b) introducing a vector comprising an HSC differentiation-inhibiting polynucleotide sequence shown in Table 1 and Table 4 into the stem cells, and (c) expressing a polypeptide encoded by the polynucleotide by culturing the modified stem cells, thereby inhibiting differentiation of the stem cells.

2. The method of claim 1, wherein the population of stem cells are isolated from bone marrow.

3. The method of claim 1, wherein the stem cells are human hematopoietic stem cells.

4. The method of claim 3, wherein the stem cells are first selected for expression of CD34 and Thy prior to introducing the vector.

5. The method of claim 1, wherein the stem cells are mouse hematopoietic stem cells.

6. The method of claim 5, wherein the stem cells are first selected for expression of CD38 and lack of expression of CD34 prior to introducting the vector.

7. The method of claim 1, wherein the HSC differentiation-inhibiting polynucleotide encodes GATA-binding protein 3 (Gata3) or ID3.

8. A method for increasing the effective dose of hematopoietic stem cells in a mammalian subject, comprising (a) providing a population of hematopoietic stem cells, (b) introducing into the cells an HSC differentiation-inhibiting polynucleotide selected from Table 1 and Table 4, and (c) administering the genetically modified cells that express an HSC differentiation-inhibiting polypeptide to a mammalian subject; thereby increasing the effective dose of hematopoietic stem cells in the subject.

9. The method of claim 8, wherein the administered stem cells are a subpopulation of the modified cells that are selected for expression of the polypeptide prior to administering to the subject.

10. The method of claim 8, wherein the administered stem cells overexpress the HSC differentiation-inhibiting polypeptide.

11. The method of claim 8, wherein the hematopoietic stem cells are obtained from bone marrow.

12. The method of claim 8, wherein the subject is human, and the hematopoietic stem cells are human hematopoietic stem cells.

13. The method of claim 12, wherein the hematopoietic stem cells are selected for expression of CD38 and Thy prior to introduction of the HSC differentiation-inhibiting polynucleotide.

14. The method of claim 8, wherein an expression vector comprising the HSC differentiation-inhibiting polynucleotide is introduced into the cells.

15. A method for inhibiting hematopoietic stem cell differentiation, comprising contacting a population of HSCs with an effective amount of an HSC differentiation-inhibiting polypeptide selected from Tables 1 and 4, thereby inhibiting differentiation of the HSCs.

16. The method of claim 15, wherein the HSCs are present in an in vitro cell culture.

17. The method of claim 15, wherein the HSCs are present in a subject grafted with the HSCs.

18. The method of claim 15, wherein the subject is human, and the HSC differentiation-inhibiting polypeptide is selected from the group shown in Table 2.

19. A method for isolating a population of cells that are enriched for hematopoietic stem cells (HSCs), the method comprising (a) obtaining a sample of cells containing hematopoietic stem cells, (b) selecting cells from the sample based on expression or lack of expression of at least one known HSC surface marker, and at least one molecule shown in Table 2 and Table 7 and (c) separating cells with the known HSC marker and at least one of the molecules shown in Table 2 and Table 7 thereby isolating a population of human cells enriched for hematopoietic stem cells.

20. The method of claim 19, wherein the hematopoietic stem cells are human HSCs.

21. The method of claim 20, wherein the known HSC marker is $CD34^+$ and Thy^+.

22. The method of claim 20, wherein the at least one molecule is a surface molecule shown in Table 2.

23. The method of claim 19, wherein the hematopoietic stem cells are mouse HSCs.

24. The method of claim 23, wherein the known HSC marker is $CD38^+$ and $CD34^-$.

25. The method of claim 23, wherein the isolated population of cells are also selected for expression of c-kit and Sca-1 but lack of expression of Lin.

26. The method of claim 19, wherein the sample of cells are obtained from bone marrow.

27. A method of enumerating hematopoietic stem cells in a population of cells, comprising (a) contacting the population of cells with an antibody that specifically binds to one HSC surface marker shown in Table 2 and Table 7 under conditions which allow the antibody to specifically bind to the HSC surface marker; and (b) quantifying the

cells recognized by the antibody; thereby enumerating hematopoietic stem cells in the population of cells.

28. The method of claim 27, wherein the population of cells is a mixture of hematopoietic cells.

29. The method of claim 27, wherein hematopoietic stem cells are human HSCs, and the population of cells are first selected for expression of CD34 and Thy prior to the contacting.

30. The method of claim 27, wherein hematopoietic stem cells are mouse HSCs, and the population of cells are first selected for expression of CD38 but lack of expression of CD34 prior to the contacting.

RV expression vector

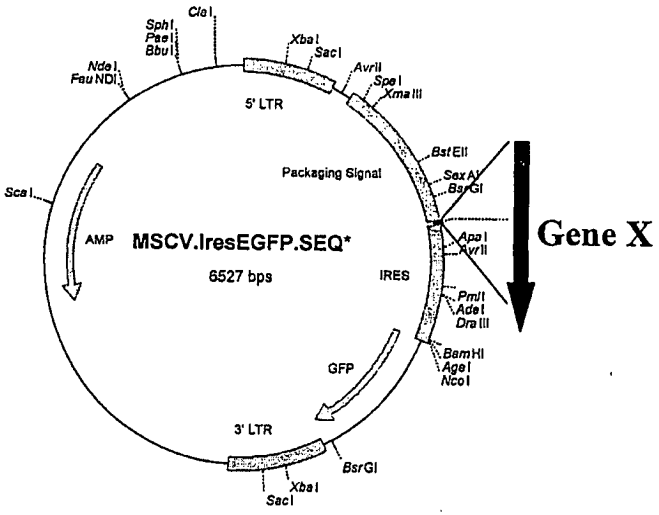
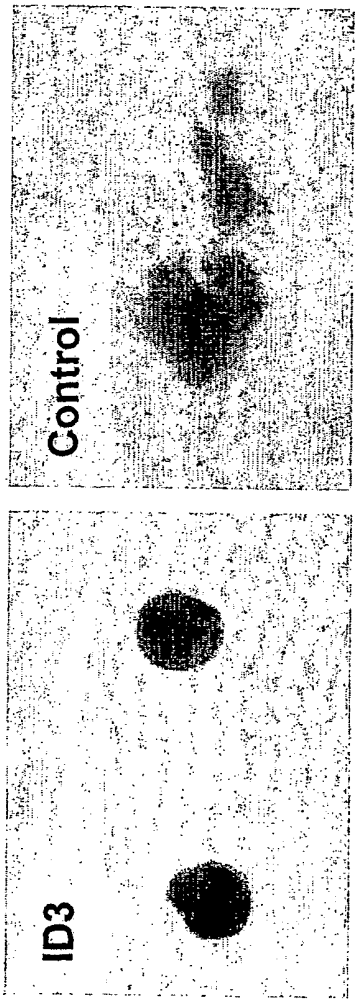


FIG. 1



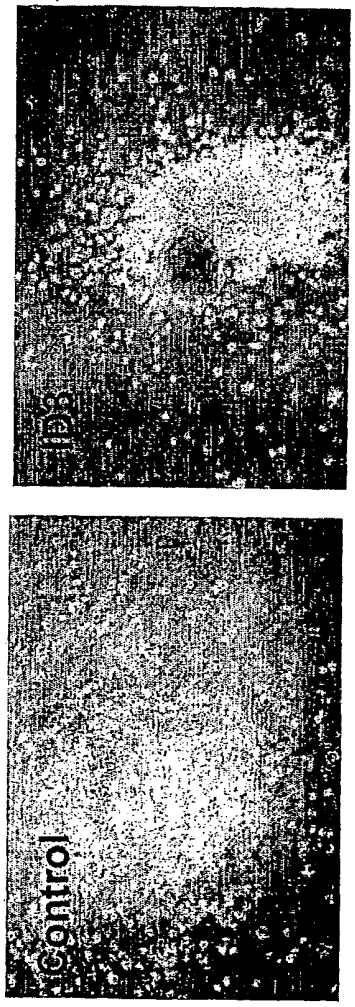


FIG. 2

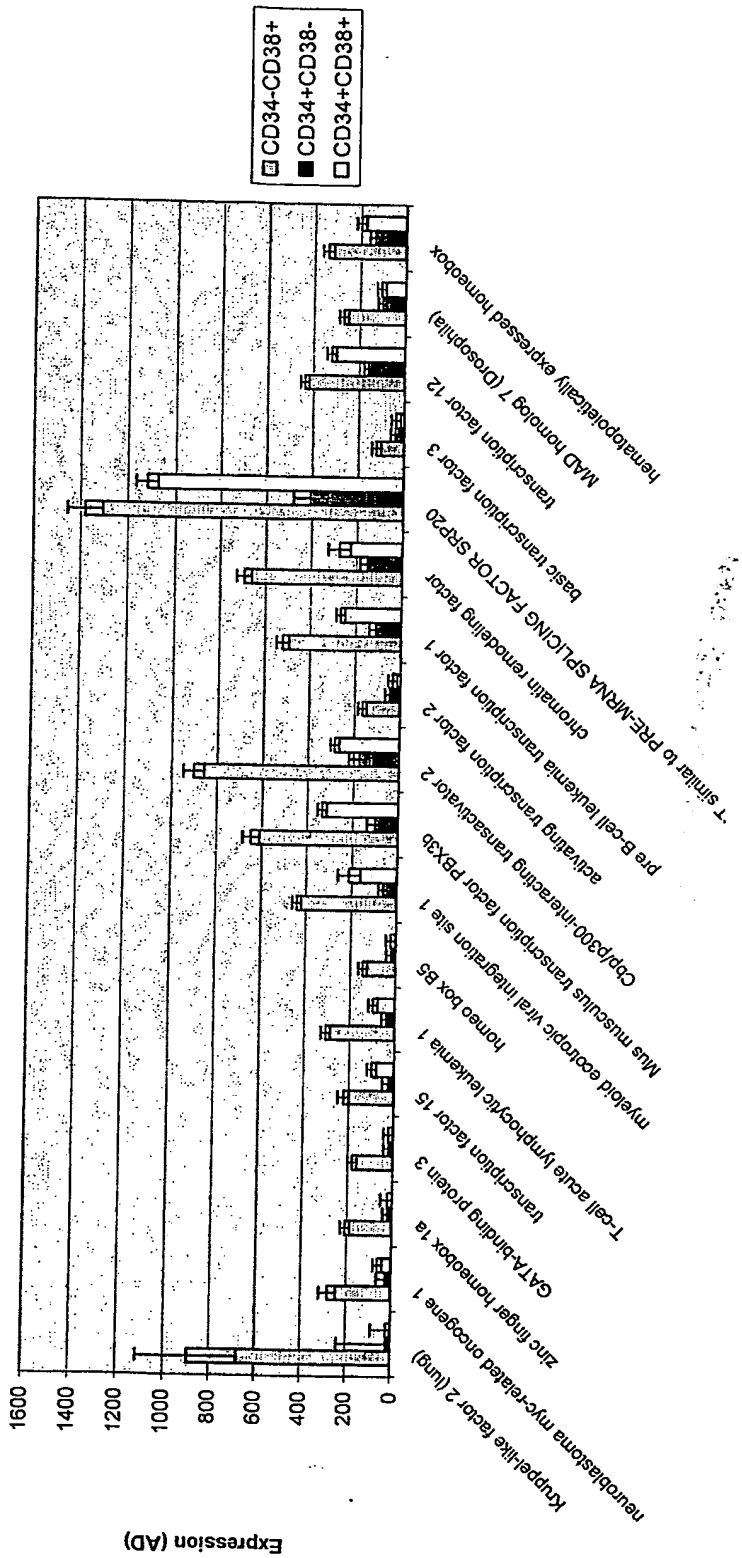


FIG. 3

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